Annual Red Imported Fire Ant Conference

Baton Rouge, Louisiana
March 21-23, 2004

LSU
AgCenter
Research & Extension

Proceedings of the

Red Imported Fire Ant Conference
March 21-23, 2004
Baton Rouge, Louisiana

<u>**Planning Committee**</u>:

Dale Pollet, Chair
Department of Entomology
LSU AgCenter

Seth Johnson
Department of Entomology
LSU AgCenter

Patricia Beckley
Department of Entomology
LSU AgCenter

Stacy Clayton
Department of Entomology
LSU AgCenter

This publication is the result of a special Red Imported Fire Ant Conference organized by the Department of Entomology at the Louisiana State University, LSU AgCenter.

The opinions, conclusions, and recommendations are those of the participants and are advisory only. Trade and company names are used in this publication solely to provide specific information. Mention of a trade or company name does not constitute a warranty or an endorsement by the LSU AgCenter to the exclusion of other products or organizations not mentioned.

The papers and abstracts published herein have been duplicated as submitted and have not been subjected to a peer review. They have been collated and duplicated solely to promote an exchange of information and may contain preliminary data not fully analyzed. For this reason, the authors should be consulted before referencing any of the information printed herein. This proceedings issue does not constitute formal peer review publication. However, ideas expressed here are the sole property of the author(s) and set precedence in that the author(s) must be given due credit for their ideas.

The 2005 IFA Conference will be held in Gulfport, Mississippi at the Grand Casino/Oasis Resort on March 22-24, 2005. The 2006 IFA Conference will be held in Alabama.

A copy of these Proceedings may be obtained for a $10.00 fee from:

Department of Entomology
Louisiana State University
LSU AgCenter
400A Life Sciences Building
Baton Rouge, LA 70803

Please write or call Dale Pollet, (225) 578-2180

General Table of Contents

ACKNOWLEDGMENTS

RED IMPORTED FIRE ANT CONFERENCE
March 21-23, 2004
Sheraton Hotel, Baton Rouge, Louisiana
Hosted by LSU Department of Entomology and LSU AgCenter

SPONSORS:
BASF
Wellmark International
RIFARID, LLC
Bengal Products, Inc.
Ambrands
Bayer Environmental Science
Diatect International

DONORS:
Zapp's Potato Chip Company
Tony Chachere's Creole Foods of Opelousas, Inc.
Chef Paul Prudhomme
McIlhenny Company
Louisiana Rice Growers Association
Dumas Candy Company

Room Information:

Area-wide Meeting	Baton Rouge East
Registration	Capitol Atrium near Baton Rouge West
Posters	Baton Rouge West
Exhibits	Capitol Atrium
Audio/Visual practice room	Baton Rouge East
Continental breakfast/refreshments	Capitol Atrium
Presentations	Iberville Ballroom A-C

Sunday, March 21, 2004

3:00 p.m.–6:00 p.m. Registration	Capitol Atrium near Baton Rouge West
3:30 p.m.–6:30 p.m. Area-wide Meeting	Baton Rouge East
3:00 p.m.–6:00 p.m. Poster Setup	Baton Rouge West
6:30 p.m.–8:30 p.m. Reception	Capitol Atrium
Crawdaddy and the Crustaceans	

Monday, March 22, 2004
7:00 a.m.–8:00 a.m. **Continental Breakfast**
7:00 a.m.–12:00 noon - **Registration**
Message Board   Outside Baton Rouge West

Moderator – Dr. Dale Pollet
8:00 a.m.–8:20 a.m. **Welcome**
            Dr. Ken Roberts, Associate Vice Chancellor and Associate Director
            Louisiana Cooperative Extension Service, LSU AgCenter

            Dr. Tim Schowalter, Head
            Department of Entomology, LSU AgCenter

8:20 a.m.–9:00 a.m. **Guest Presentation**
            Title: **A Fire Ant Odyssey**
            Mr. Ed Bordes, Director
            New Orleans Termite & Mosquito Control Board

Eradication Efforts:

9:00 a.m.–9:12 a.m. **RIFARID LLC's New One-Step Method for Instant Eradication of RIFA Colonies**
Author: Tommy G. Taylor, RIFARID LLC

9:12 a.m.–9:24 a.m. **Prevention Efforts in Hawaii against RIFA**
Author: Neil J. Reimer, Hawaii Department of Agriculture

9:24 a.m.–9:36 a.m. **RIFA Plans in the Pacific**
Authors: Carol Russell, USDA-APHIS, PPQ and
Neil Reimer, Hawaii Department of Agriculture

Regulatory Issues & Quarantine:

9:36 a.m.–9:48 a.m. **Contact Insecticide Treatments Applied to Fire Ant Mounds during Winter Months as Potential Nursery Quarantine Treatments-2003 Mississippi**
Authors: Shannon S. James, Anne-Marie Callcott, Jason B. Oliver,
Nadeer N. Youssef, and Karen M. Vail, Tennessee State University

9:48 a.m.–10:00 a.m. **Pesticides Applied to Individual Fire Ant Mounds during Winter Months as Potential Treatments for Certifying Nursery Stock-2003 Tennessee Trial**
Authors: Jason B. Oliver, Shannon S. James, Ann-Marie Calcott,
Karen M. Vail, Nadeer N. Youssef, Tennessee State University

Chemical Control:

10:00 a.m.–10:12 a.m. **Advances in Imported Fire Ant Bait Technology: Extinguish®R Plus, a Blend of Methoprene and Hydramethylnon, and the Modified Herd G-77 Model Air-Assisted Applicator**
Authors: Bastiaan "Bart" M. Drees, Texas A&M University
Doug VanGundy, Wellmark International
David Herd, Herd Seeder Company

10:12 a.m.–10:24 a.m. **How Fast is Fast?: Indoxacarb Broadcast Bait**
Author: Charles L. Barr, Texas Cooperative Extension

10:24 a.m.–11:00 a.m. **Break   Posters/Exhibits**

11:00 a.m.–11:12 a.m. **Lago Santa Fe Fire Ant Project, Santa Fe, Texas: The Never Ending Story!**
Authors: Paul R. Nester, Corrie P. Bowen, Bastiaan "Bart" M. Drees,
Texas Cooperative Extension

11:12 a.m.–11:24 a.m. **Laboratory Evaluations of Liquid Toxicants against Red Imported Fire Ants**
Authors: Beverly A. Wiltz , Daniel R. Suiter, and Wayne A. Gardner
University of Georgia

11:24 a.m.–11:36 a.m. **A New Method for Evaluating Chemical Repellency in *Solenopsis invicta* Buren *(Hymenptera: Formicidae)***
Author: Jian Chen, USDA-ARS

11:36 a.m.–1:00 p.m. **Lunch**
1:00 p.m.–2:00 p.m. **Posters/Exhibits**

Moderator – Dr. Linda Hooper-Bui

Biology and Ecology:

2:00 p.m.–2:12 p.m. **Stimulation of Black Imported Fire Ants (*Solenopsis richteri* Forel) Using Substrate-borne Vibrations and the Effects on the Parasitism of Host Ants by Attacking Phorid Flies (*Pseudacteon curvatus* Borgmeier)**
Authors: Esther Mwangi, Roger Hasse, Paul Lago, and Richard Buchholz
University of Mississippi
Douglas Streett, USDA/ARS-BCMRRU

2:12 p.m.–2:24 p.m. **Effects of Fire Ants on Peanut Plants**
Authors: Roger E. Nelson, Carl Albert State College
Stanley A. Rice, Southeastern Oklahoma State University
J.T. Vogt, USDA/ARS-BCPRU

2:24 p.m.–2:36 p.m. **Diurnal Patterns of Ovipositional Activity in Two *Pseudacteon* Parasitoids (*Diptera: Phoridae*) in Alabama**
Authors: V.E. Bertagnolli and L.C. "Fudd" Graham, Auburn University

2:36 p.m.–3:12 p.m. **Break   Posters/Exhibits**

3:12 p.m.–3:24 p.m. **Interactions between the Pyramid Ant (*Dorymyrmex flavus* McCook) and the Red Imported Fire Ant**
Authors: Alejandro Calixto, Charles Barr, and Marvin Harris
Texas A&M University

3:24 p.m.–3:36 p.m. **Assessment of Landscape-level Impacts of Red Imported Fire Ants on Native Invertebrate Communities in Pine-dominated Forests**
Authors: Keri E. Landry, Linda M. Hooper-Bui, Michael J. Chamberlain, and Lee A. Womack, LSU Agricultural Center

3:36 p.m.–3:48 p.m. **Effect of Fire Ant Presence in Mammal Traps on Bait Theft and Trap Success in Two Ecosystems in Louisiana**
Authors: Linda M. Hooper-Bui, Michael J. Chamberlain, and J. Constible
LSU Agricultural Center

Bio-Control:

3:48 p.m.–4:00 p.m. **Discovery and Characterization of Viruses in the Imported Fire Ant, *Solenopsis invicta***
Author: Steven Valles, USDA/ARS

4:00 p.m.–4:12 p.m. **Infection of Red Imported Fire Ant Colonies with the *Microsporidium Vairimorpha invictae***
Authors: David H. Oi, Juan A. Briano, and David F. Williams
USDA/ARS-CMAVE

4:12 p.m.–4:24 p.m. **The Evaluation and Release of Three New Fire Ant Decapitating Flies**
Authors: Sanford D. Porter and Ricardo J. Vazquez, USDA/ARS-CMAVE
Juan A. Briano and Luis A. Calcaterra, USDA/ARS-SABCL

4:24 p.m.–4:36 p.m. **Occurrence and Distribution of** *Thelohania solenopsae* **in Louisiana Red Imported Fire Ant** *(Solenopsis invicta)* **Populations**
Authors: Maynard L. Milks, James R. Fuxa, and Arthur R. Richter
LSU Agricultural Center

4:36 p.m.–4:48 p.m. **Variability in the Occurrence of** *Thelohania solenopsae* **in** *Solenopsis invicta* **Population in Oklahoma**
Authors: Vedham Karpakakunjaram and Russell E. Wright
Oklahoma State University

4:48 p.m.–5:00 p.m. **Mammalian Cardiovascular & Neurologic Responses Elicited by Synthetic Alkaloids from** *Solenopsis invicta* **(Imported Fire Ant) Venom**
Authors: George Howell, Jeremy Gibson, David McClendon, D. Nanayakkura, G-B Yi, and Robin Rockhold, University of Mississippi Medical Center and University of Mississippi

5:00 p.m.–5:12 p.m. **A New Bioassay for Evaluating Fire Ant Repellants**
Author: Jian Chen, USDA/ARS

5:12 p.m. –5:24 p.m. **Some Methods to Rear Special Colonies of Fire Ants for Special Reasons**
Authors: S. Bradleigh Vinson, Sherry Ellison, and M. Bashir
Texas A&M University

Adjourn
Dinner – on your own

Tuesday a.m., March 23, 2004
7:00 a.m.–8:30 a.m. **Continental Breakfast**

Moderator – Dr. Roberto M. Pereira
Area-wide Suppression Project:
8:30 a.m.–8:42 a.m. **Final Report on Fire Ant IPM Study on DOD Facilities in South Carolina**
Author: David F. Williams, USDA/ARS-CMAVE

8:42 a.m.–8:54 a.m. **Area-wide Fire Ant Suppression in Florida**
Author: Roberto M. Pereira, USDA/ARS

8:54 a.m.–9:06 a.m. **Phorid Fly Range Expansion and Mound Suppression in a Heavily Infested Area**
Authors: Charles L. Barr and Alejandro A. Calixto, Texas Cooperative Extension

9:06 a.m.–9:18 a.m. **Release of** *Pseudacteon curvatus* **in South Carolina**
Author: Timothy Davis, Clemson University

9:18 a.m.–9:30 a.m. **2003 Update on the Area-wide Fire Ant Management Program in Oklahoma**
Authors: Russell Wright and Vedham Karpakakunjaram
Oklahoma State University
Wayne Smith, Oklahoma Cooperative Extension Service

Other:

9:30 a.m.–9:42 a.m. **Ecologically Benign Method for Fire Ant Control**
Author: R.E. Driscoll, Sr., Driscoll Products
Hugh M. Ettinger, Hugh Ettinger Associates, Ltd.
M. Dale Mayes, BYE ANT!

9:42 a.m.–9:54 a.m. **Detection of Fire Ant Mounds in Airborne Digital Images: Hierarchical Learning for Automatic Feature Extraction**
Author: James T. Vogt, USDA/ARS-BCPRU

9:54 a.m.–10:06 a.m. **Fire Ant Attacks on Patients in Nursing Homes: An Increasing Problem**
Authors: Robin Rockhold, R.D. deShazo, S.F. Kemp, M.D. deShazo, J. Goddard
University of Mississippi Medical Center and Mississippi Department of Health

10:06 a.m.–10:30 a.m. **Break**
10:30 a.m.–11:30 a.m. **Business Meeting**
11:30 a.m. – **Adjournment**

Fire Ant Committee:
Dr. Dale Pollet
Ms. Linda Forester
Ms. Barbara Kellum
Ms. Patty Beckley
Ms. Daravanh Weeks
Dr. Seth Johnson
Dr. Linda Hooper-Bui
Dr. Lane Foil
Dr. Jack Baldwin
Dr. Jim Fuxa
Dr. Tim Schowalter
Ms. Stacy Clayton

List of Poster Presentations

1. **Survey of the Ants of Alabama.** Jason A. Forster and Michael L. Williams. Auburn University

2. **Distribution of Imported Fire Ant Populations in Alabama.** L.C. "Fudd" Graham, V.E. Bertagnolli, and R.K. Vander Meer. Auburn University

3. **Dispersal of the Decapitating Fly, *Pseudacteon Tricuspis* Borgmeier, a Biological Control Agent of the Red Imported Fire Ant, *Solenopsis Invicta* Buren in Southeast Louisiana.** D.C. Henne, S.J. Johnson. Louisiana State University, LSU Agricultural Center. S.D. Porter, USDA-ARS-CMAVE.

4. **Pesticide Surface Treatments Applied to Fire Ant Mounds in April as Potential Nursery Quarantine Treatments-2003 Tennessee Trials.** Karen Vail. University of Tennessee

5. **Phorid Fly Detection Enhancement with a Modified Electric Livestock Prod.** Charles L. Barr and Alejandro A. Calixto. Texas Cooperative Extension

6. **Confinement-Tray Color Affects Parasitism Rates of Attacking Pseudacteon curvatus (Diptera: Phoridae) in a Laboratory Rearing System.** Larry G. Thead and Douglas A. Streett. USDA, ARS Biological Control of Pests Research Unit, Stoneville, MS 38776

7. ***Myrmicinosporidium durum*: A New Fire Ant Pathogen.** Roberto M. Pereira. USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology

8. **Distribution of *Thelohania solenopsae* in Red Imported Fire Ant Populations in Mississippi.** D.A. Streett, Thomas Barton Freeland, Jr., and Anthony Pranschke, USDA-ARS-BCPRU.

9. **Insect Diversity Patterns in *Solenopsis Invicta* Infested Areas in Oklahoma.** Vedham Karpakakunjaram, Richard A. Grantham, and Russell E. Wright. Oklahoma State University

10. **A Downsized System for Rearing Phorid Flies.** Lee J. Eisenburg. Louisiana State University, LSU Agricultural Center

11. **Release of *Pseudacteon curvatus* Into Black and Hybrid Imported Fire Ant Populations in Northern Alabama, Southern Tennessee, and Eastern Mississippi.** Ken Ward and Rufina Ward. Alabama A&M University. L.C. "Fudd" Graham, Auburn University. Larry Thead and James T. Vogt. USDA-ARS, BCPRU

12. **Host and Parasite Interactions between the Red Imported Fire Ant and a Microsporidian Parasite.** M. Walker Hale and S.B. Vinson. Texas A&M University

13. **PCR-based Analysis of Spores Isolated from Smears by Laser Pressure Catapult Microdissection Techniques Confirms Genetic Identity of Spore Morphotypes of *Thelohania solenopsae*.** Yuliya Y. Sokolova, Lacey R. McNally, and James R. Fuxa. Louisiana State University, LSU Agricultural Center

14. **Evaluation of Broadcast Applications of Cyfluthrin and Beta-cyfluthrin Against Red Imported Fire Ants (Solenopsis invicta).** Kelly Loftin, John Hopkins, Doug Petty, and Donna Shanklin. UA-CES

15. **Evaluation of Selected Insecticides as Individual Mound Treatments Against Red Imported Fire Ants.** Kelly Loftin, John Hopkins, Allan Beuerman. UA-CES

16. **Cleveland County, Arkansas - Neighborhood Demonstration of Fire Ant Management.** Les Walz, Donna Shanklin, Kelly Loftin, and John Hopkins. UA-CES

17. **Calhoun County, Arkansas– 3 Communities, 3 Fire Ant Management Efforts.** Alan Lee, Donna Shanklin, Kelly Loftin, and John Hopkins. UA-CES

18. **Kid₂Ants®: A New Curriculum about Fire Ants for Kids.** Nathan Riggs, Elizabeth Brown, Paul Nester. Texas Cooperative Extension

19. **Interstate Collaborative Efforts to Develop Educational Programs for Fire Ant Management in Cattle Production Systems.** Flanders, K.L. and B.M. Drees.

20. **RIFA, *Solenopsis invicta* Management in a South Louisiana Citrus Orchard.** Dale Pollet, Patricia Beckley, and Boris Castro, Louisiana State University, LSU AgCenter

21. **Initiation of Flight Muscle Histolysis in the Red Imported Fire Ant (*Solenopsis invicta*)** T. Azizi and S.B. Vinson. Texas A&M University

22. **Gene Overexpression Associated with Workers of the Red Imported Fire Ant, *Solenopsis invicta* Buren.** Nannan Liu and Lee Zhang. Auburn University

Index to Speaker Abstracts

Eradication Efforts

RIFARID LLC's NEW ONE-STEP METHOD FOR INSTANT ERADICATION OF RIFA COLONIES

Tim Taylor
Business Development Manager
RIFARID LLC
4011 Creole Street
Lake Charles, LA 70605
Phone: 337-477-8190 or 303-641-5389
E-Mail: info@rifarid.com

RIFARID LLC is a family-owned Louisiana company established for the purpose of commercializing a pending patent that we believe represents the fastest, safest, most effective method ever devised for eradicating the Red Imported Fire Ant (RIFA). Our invention teaches a novel fire ant mound treatment method involving the use of a volatile, non-toxic chemical agent that possesses the ability to knock out instantly an entire colony of these hardy insect pests. We have trade-named our preferred knock-out agent RIFARIDTM. Within seconds, a relatively minute quantity of RIFARID™ will put an entire fire ant colony to sleep for one to two hours. We have tested RIFARID™ in combination with numerous common insecticides including chlorpyrifos, acephate, diazinon, esfenvalerate, cyfluthrin, lamda-cyhalothrin, deltamethrin and d-limonene. Our tests reveal that most of these poisons achieve relatively limited efficacy by themselves. However, in combination with RIFARID™ we have determined that even the weakest and shortest-lived of these insecticides will completely and permanently eradicate a targeted fire ant colony.

Tom Taylor, founder and president of RIFARID LLC, invented and perfected the RIFARID™ fire ant eradication method. Mr. Taylor retired from PPG Industries of Lake Charles, Louisiana in 1997 after a productive 41-year career as a chemical engineer, during which time he worked in all aspects of PPG's chlorinated hydrocarbon business. Among many other chemical manufacturing operations, Tom Taylor played a major role in the development and commercialization of a certain compound called trans-1, 2-dichloroethylene. He led PPG's effort to produce and market this product commercially.

Trans-dichloroethylene is the preferred knock-out agent contained in our RIFARID™ product. The EPA approved trans-dichloroethylene for emissive use applications in the 1980's, and on August 26, 1994 the compound appeared in the Federal Register as an environmentally acceptable alternative solvent for emissive use applications. Trans-dichloroethylene is used today primarily in aerosol formulations for cleaning electronic components. It represents a particularly desirable replacement for a number of other volatile organic compounds due to its ease of manufacture, its extremely low toxicity and its zero ozone-depletion potential. The compound is not a carcinogen. Extensive toxicity tests sponsored by DuPont, PPG and 3M in 1999 determined that trans-

dichloroethylene possesses exceptionally attractive NOEL and LD-50 ratings. Additionally, we performed tests at McNeese State University under the direction of Dr. Ron Darbeau, Chairman of the McNeese Chemistry Department, which tests decisively prove that RIFARID™ poses no threat to ground water contamination when applied according to the method taught in Tom Taylor's invention. We have also recently begun the application process to gain EPA approval for the RIFARID™ method.

For all of these reasons, RIFARID LLC considers trans-1,2-dichloroethylene an excellent knock-out agent for its Red Imported Fire Ant eradication method. We also believe it will prove equally effective for eradicating other nesting insect pests such as the Formosan termite or the yellow jacket wasp. As long as the targeted insect pest is housed within a confined space, vapors from the highly volatile trans-dichloroethylene will permeate the cavities of the nest and then readily evaporate and dissipate into the atmosphere. This is proven by the fact that a targeted fire ant nest, when exposed to RIFARID™ alone, will completely recover within minutes or hours once the trans-dichloroethylene vapors have dissipated.

The RIFARID LLC treatment method is simple and fast, making it ideal for home consumer use. One only needs a one or two-gallon receptacle for holding the RIFARID™ -poison-water solution, a stirring implement for mixing the solution and a slender shaft for puncturing holes into the targeted fire ant mound. We have preferred using a sprinkler can as our vessel for mixing and pouring the RIFARID™ -poison-water solution. Into this sprinkler can we add a prescribed amount of RIFARID™ knock-out solution, a prescribed amount of any of several general purpose insecticides according to manufacturer specifications, and then complete the solution with a prescribed amount of water. A one-gallon volume is particularly convenient to manage and is usually sufficient to eradicate a typical fire ant colony found in a residential area (up to 12 inches in diameter). We then agitate the solution with our mixing implement, forming an emulsion of RIFARID™, water and the selected poison.

Next, we thoroughly wet the surface of a targeted RIFA mound. In order to ensure that the poison reaches the ants deep within the nest, including the all-important queens, eggs and larvae, we then puncture several holes into the mound to a depth of one to three feet, depending upon the size of the mound, and then we pour the remainder of the solution into the punctured mound. However long it takes a selected poison to kill a fire ant will determine how soon the colony will die. At this stage you will observe the powerful effect of the RIFARID™ knock-out solution as all swarming activity ceases. You may observe a few individual ants twitching upon the surface of the mound, for these ants are not trapped within cavities permeated by the trans-dichloroethylene vapors. However, you will not observe any ants emerging from within the mound.

We routinely excavate our targeted test mounds about 24 hours after application of the RIFARID™ -poison-water solution, and we have always found abundant clusters of dead ants, eggs and larvae within these excavated mounds. We have never observed the revival of a targeted mound, and most importantly we have never observed the formation of new mounds adjacent to a targeted and properly treated mound, as we often, if not usually observe in tests employing only a dry poison or a poison-water solution. As I mentioned earlier, our tests reveal that any number of safe, garden insecticides will work with complete efficacy when married to our RIFARID™ knock-out solution.

We like to describe our home consumer procedure as the "*M-Triple-P technique*" ("*mix, pour, puncture and pour*"). When properly executed, the *M-Triple-P technique* will instantly immobilize, trap and kill every ant, larva and egg within the confines of a targeted RIFA colony. No ants will escape to start new colonies elsewhere, and the targeted colony will be dead forever. In short, ours is a genuine "wet-it and forget-it" procedure.

RIFARID LLC has tested very large RIFA mounds, such as you might typically find in a cattle pasture or in other areas where ants multiply unmolested by mowers or other human activity. Although greater volumes of RIFARID™ -poison-water solution are required for very large mounds (ex. Approximately 3 gallons of RIFARID™ -poison-water solution for a mound two feet in diameter), our eradication method works impressively well, despite the size of the mound. When applied systematically and aggressively, the RIFARID™ method represents the safest, most viable way to reclaim pasture and farm acreage lost to heavy RIFA infestation.

We believe that blanketing farm and pasture lands with lingering poisons does not represent a viable method for eradicating RIFA infestations. These lingering poisons may imperil our very food supplies and may pose a significant threat to ground water and to other non-target insect and animal species. Therefore, we are developing mechanized procedures for adapting the RIFARID™ *M-Triple-P* technique to the treatment of rampant infestations over substantial acreage. The RIFARID™ method can be employed to reclaim the hundreds of thousands of acres rendered useless due to heavy RIFA infestation.

In closing, we must acknowledge the obvious: the Red Imported Fire Ant problem is not improving. Despite the best efforts heretofore manifested by the scientific community, the RIFA continues to spread unabated, reaching genuinely epidemic proportions in this country. Over the last seventy years, this virulent non-indigenous pest, *solenopsis Invicta*, has spread across the entire Southern half of the United States, imperiling our yards and gardens, our parks, our playgrounds and athletic fields, and some of the choicest farm and pastureland in the world. Certain bird and animal species, such as the gopher tortoise, the quail and the highly endangered Atwater prairie chicken have virtually disappeared due to competition and predation by the Red Imported Fire Ant.

Controlling the fire ant is not the solution. Eradication is the solution. RIFARID LLC possesses the method to accomplish this single viable goal. We encourage you to contact us and to visit our booth to learn more about us. We have an engaging video display that proves the efficacy of the RIFARID™ method. We are anxious to discuss with you our commercialization efforts and needs. We would like to arrange tests of our method with certain parties attending the conference, and we are anxious to explore business opportunities, perhaps finding the best poison to which we might wed our RIFARID™ treatment method. That poison, in combination with RIFARID™, can become the be-all and end-all of fire ant eradication in the United States. We seek partners and advocates for this exciting and effective method for RIFA eradication, and we encourage all interested parties to consider the extraordinary commercial opportunities our method provides.

Prevention Efforts in Hawaii Against **Red Imported** Fire Ant.
By: Neil Reimer, Hawaii Department of Agriculture

Hawaii is a remote chain of islands separated from the nearest continental land mass by approximately 2,500 miles. As a result, the Hawaiian fauna is devoid of native ants. All of the **46** species of ants established in Hawaii were transported to the islands through human activities. The majority of these are tramp species and are restricted to the lower elevations below 1,200 meters. Many of the ant species that reached Hawaii have had a severe impact on native arthropod populations as these were not adapted to ant predation.

Hawaii is greatly concerned with the movement of RIFA across the continental U.S. and particularly with its entry into California. The vast majority of nursery stock and other items arriving in Hawaii are transported by air or ship from California. The Hawaii Department of Agriculture (HDOA) has a policy to reject or treat any shipment infested with ants if the species is not present in Hawaii and has stages capable of reproduction. These conditions include, queens present, workers present with egg, larvae, or pupae, workers present and 100% inspection is impossible, or a gamergate species. HDOA and the Hawaii Ant Group (HAG, a multiagency working group composed of government agencies and **NGO's**) has worked with USDA to modify the USDA ant interdiction policy. USDA now has a policy similar to **HDOA's** for interceptions of material coming to Hawaii.

HDOA conducted a pathway risk analysis for the movement of RIFA to Hawaii. In addition, HAG has developed a RIFA action plan composed of prevention, detection, response, enforcement, and public outreach components. HDOA developed a prevention protocol for the high-risk pathways and is conducting surveys for RIFA at ports-of-entry, such as airports, harbors, and nurseries.

Carol Russell

United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA/ APHIS/ PPQ) 300 Ala Moana Boulevard, Room 8-152, P.O. Box 50002, Honolulu, HI 96850

Red Imported Fire Ant: Prevention "Plans" in the Pacific

Addressing efforts in Hawaii and throughout the Pacific region, requires better prevention measures for possible incursions of red imported fire ants. Hawaii and the Pacific Island Countries and Territories (PICT) are known for their island isolation and fragile ecosystem. An incursion of red imported fire ants could spread rapidly and go undetected. Detection in Hawaii is done through survey programs through funds provided by USDA/ APHIS/ PPQ to the Hawaii Department of Agriculture. The Hawaii Ant Group, established in 1999, provides a comprehensive and cooperative approach to deal with new ant threats. They have created a state prevention plan, provided the information for a change in quarantine policy for ants intercepted from commodities destined to the State of Hawaii, acquired funds, and participated in a worltshop in New Zealand to initiate a regional approach to prevention. In October 2002, the Global Invasive Species Program (GISP) convened in Hawaii. Participants from PICT were in unanimous agreement that RIFA seem to pose a huge threat to Pacific island biodiversity, island economies and culture, and human health. A workshop held by the Invasive Species Specialist Group (ISSG) in Auckland, New Zealand in September 2003, resulted in the compilation of a draft Pacific Ant Prevention Plan (PAPP) that encompassed RIFA and other exotic invasive ants that have demonstrated negative impacts. Given the eradication difficulties of many invasive alien ant species (esp RIFA) obviously prevention of entry is of utmost importance and the highest priority. Given many preventative biosecurity measures may take time to implement on a regional basis the PICT region also need to be prepared for an incursion. The PAPP proposal outlines the necessary framework for both the prevention of entry and the prevention of establishment. As of March 2004, the Pacific Plant Protection Organization and Regional Technical Meeting for Plant Protection was held in Suva, Fiji. The Pacific Ant Group which worked on the plan presented this plan at the meeting.

Phone: (808) 541-1980
FAX: (808) 541-1978
Email: carol.e.russell@aphis.usda.gov

Regulatory Issues & Quarantine

CONTACT INSECTICIDE TREATMENTS APPLIED TO FIRE ANT MOUNDS DURING WINTER MONTHS AS POTENTIAL NURSERY QUARANTINE TREATMENTS – 2003 MISSISSIPPI

Shannon S. James[1], Anne-Marie Callcott[1], Jason B. olive?, Nadeer N. Youssef[2], and Karen M. Vail[3]

[1] USDA, APHIS, PPQ, Soil Inhabiting Pests Laboratory, Gulfport, MS
[2] Tennessee State University, Institute for Agricultural and Environmental Research, Otis L. Floyd Nursery Research Center, McMinnville, TN
[3] University of Tennessee, Department of Entomology and Plant Pathology, Knoxville, TN

ABSTRACT

Simulating treatment of infested individual in-field B&B nursery product, several contact insecticides were applied to fifteen 36 inch (0.914 m)-diameter circular plots containing active fire ant mounds. Fire ant nests were checked weekly for activity through seventeen weeks after treatment or until treatment failure. Colonies in all plots treated with deltamethrin (0.13 lb a.i./A) and lambda-cyhalothrin (1.76 lb a.i./A) were inactive within two weeks of treatment and remained inactive until the ten week post-treatment observation. Bifenthrin flowable (0.20 lb a.i./A) and lambda-cyhalothrin (0.88 lb a.i./A) displayed control in all but one plot of each at two weeks after treatment. These two treatments attained 100% control in weeks 4-10 and 5-8, respectively. After rain between observations at week 4 and 5, the number of active bifenthrin granular (0.20 lb a.i./A) treated plots dropped from thirteen to one. The dry and the water treated control treatments ended the trial with 14 and 7 active colonies respectively, indicating that treatments with no active colonies provided control by means of chemical activity and not the disturbance of treatment alone. Treatments with acceptable results as reported in this trial will undergo further testing both by the APHIS lab in Mississippi and our cooperators in Tennessee until new quarantine treatments are determined.

INTRODUCTION

The U.S. Dept. of Agriculture's Animal and Plant Health Inspection Service (APHIS) is responsible for developing treatment methodologies for certification of regulated items, such as field-grown balled-and-burlapped (B&B) nursery stock, in the Imported Fire Ant Quarantine (7CFR 301.81). Imported fire ants are slowly moving into areas of Tennessee where many producers of field grown nursery stock are located. Approximately 84% of the plants from this area are shipped to states or locations within states outside the quarantine (Brooker et al. 2000). This has prompted a renewed interest in development of new treatments for this stock.

Currently the Federal Imported Fire Ant Quarantine has three treatment regimens available for movement of B&B nursery stock. Harvested B&B may either be immersed in a chlorpyrifos solution until bubbling ceases, or drenched to the point of runoff twice daily for three consecutive days with a chlorpyrifos solution. B&B plants not yet harvested (in-field stock) may be certified for shipment by applying an approved bait followed in 3-5 days by an application of granular chlorpyrifos. Application of the in-field B&B treatment must extend a minimum of 10 ft from the base of the plants to be shipped out of the quarantine area. Plants treated in this manner are certified for shipping 30 days after the application of the granular chlorpyrifos.

Aside from the reliance on a single chemical, all three application methods are not well received by growers for various reasons. Immersion treatments require specialized equipment, produce large amounts of waste chemical, and sometimes cause loss of plants through phytotoxicity and loss of root ball integrity. Both the immersion and drench options are labor intensive and pose exposure risks for handlers. The in-field treatment reduces exposure risks and produces no waste to be disposed of, but the 10 ft minimum radius has growers often applying this treatment to plants a row or more on either side of what is actually being harvested. B&B plants are generally harvested in cold weather when plants are dormant, so the in-field treatment's need for active IFA foraging on baits coupled with the 30-day exposure period mean that all potential shipments out of IFA quarantined areas must be anticipated more than a month in advance.

In a collaborative effort personnel from the APHIS Soil Inhabiting Pests Laboratory, Tennessee State University (TSU), and the University of Tennessee (UT) currently are running a battery of experiments designed to examine new methods of application and chemical alternatives for inclusion in IFA quarantine treatments for B&B. Goals specifically for new in-field treatments are addition of other chemicals, reduction of treatment radius, and a reduction in pre-shipment exposure period. The trial reported herein attempts to address these goals by examining spot treatments of simulated in-field infestation.

Numerous formulations of common insecticides such as diazinon, chlorpyrifos, acephate, and others are labeled for spot treatment of imported fire ant colonies. Imported fire ant colonies readily respond to insecticide applications made directly to the nest by relocating the colony (Collins & Callcott 1995, Hays et al. 1982, Franke 1983, Williams & Lofgren 1983). The primary objective of a quarantine treatment for B&B nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed by the treatment or simply induced to move away from the area around each individual plant intended for harvest.

Previous spot-treatment trials initiated by APHIS in Mississippi in January and September 2001 assessed several liquid and granular insecticides against individual IFA mounds in the field. Results of these trials indicated promising results with acephate, bifenthrin, and deltamethrin products. A similar trial conducted February through March of 2001 by TSU cooperators indicated that lower temperatures experienced at the Tennessee test site might have hindered the performance of some of the treatments.

Since B&B nursery stock is generally harvested in the winter product performance in low temperatures is of concern. Thus treatments in this trial were applied and observation initiated in January 2003 by APHIS and February 2003 by TSU. A small-scale warm weather trial was also conducted by cooperators at UT. Both sets of trials conducted in Tennessee are reported elsewhere in the fire ant conference proceedings.

MATERIALS AND METHODS

Test plots consisted of individual IFA mounds and the surrounding ground that fit within a 36" (0.914 m)-diameter circle. This size plot represents the smallest commonly harvested root ball size, 12" diameter, plus a 12" treated buffer zone surrounding the area to be harvested. One hundred twenty IFA mounds in a pasture in Harrison Co., MS were marked for use and divided into fifteen replications for each treatment. Mound activity was determined by poking a wire flag in the mound and observing IFA response. Wooden stakes labeled with the plot identification number were planted in close proximity to each plot to aid in visually locating the plot and attributing results to the appropriate treatment. Hula-hoops with a 36" diameter were utilized in conjunction with orange spray paint to uniformly mark the treatment areas around each plot.

Treatments were applied and plot observation initiated January 16, 2003. A roller pump-powered 55-gallon spray tank with an adjustable garden nozzle set for shower pattern attached to a garden hose was used to apply liquid treatments. Two gallons of the appropriate liquid treatment, including the water control, were applied on each of their respective plots. Preweighed amounts of the granular treatment were packed in individual zip-close bags and sprinkled over plots by hand. The treatments in this trial are as follow:

Product	Active Ingredient	Rate of Application
Talstar PL – granular	bifenthrin	0.20 lb a.i./acre
Talstar Lawn & Tree – flowable	bifenthrin	0.20 lb a.i./acre
Scimitar CS	lambda-cyhalothrin	0.88 lb a.i./acre
Scimitar CS	lambda-cyhalothrin	1.76 lb a.i./acre
DeltaGard 5SC	deltamethrin	0.13 lb a.i./acre
Sevin SL	carbaryl	4.00 lb a.i./acre
Wet control	---	Water only
Dry control	---	No application

Observations of mound activity within the plots were conducted weekly after application until seventeen weeks had passed or failure of treatment. Temperature data throughout the duration of the trial was collected using a StowAway® data logger and accessed using BoxCar® 3.6 (Onset computer Corp., Bourne, MA). Additional readings of air and soil temperatures using the appropriate thermometers were recorded along with total precipitation during weekly plot evaluations.

RESULTS AND DISCUSSION

The deltamethrin, both rates of lambda-cyhalothrin, and the bifenthrin flowable treatments delivered fast knock down in their respective plots (Figures 1 and 2). All colonies in the deltamethrin and high rate of lambda-cyhalothrin were inactive by two weeks after treating and remained inactive until observation at ten weeks after treatment. Bifenthrin flowable and the lower rate of lambda-cyhalothrin displayed 100% inactivity in their plots in weeks 4-10 and 5-8 observations respectively. There was no evidence of product efficacy in the granular bifenthrin treatment until after rain showers in the trial area between week four and five observations (Figure 3). It appears that the granular bifenthrin treated plots may have been prone to reinfestation while still maintaining some insecticidal activity, as a different plot was infested each week after the week six observation. Likewise when renewed activity was seen in the flowable bifenthrin and high rate lambda-cyhalothrin treatment plots, it was never in the same plot twice. At two months after application the carbaryl treatment had three plots that never became inactive and one that was reinfested. This treatment was only slightly more effective at removing active fire ant colonies than water. Carbaryl, after the week eight observation, was determined to be inadequate to quarantine needs and further observation of those plots was terminated. When the trial was terminated, 17 weeks after treatment application, the untreated dry control had only lost activity in one of its plots. The water treated control demonstrated a relatively slow descent to a minimum of five active plots but rebounded to seven by the trial's end.

The loss of activity seen among the water treated plots compared to the untreated control plots is a testament to the ability of disturbance to move IFA colonies. However, the water treatment and the carbaryl treatments both did not reach levels considered adequate for quarantine purposes (zero tolerance) indicating those treatments that did reach a point with no active colonies did so by either insecticidal or repellant properties of the product. Trials initiated during winter 2003 in both Mississippi and Tennessee (reported separately in the conference proceedings) indicated that chemical applications of deltamethrin, lambda-cyhalothrin, and flowable bifenthrin merited further consideration. Replication of this test by APHIS and TSU is currently underway at both locations. The trial (reported separately in the conference proceedings) conducted in Tennessee in spring and summer 2003 indicated efficacy of these treatments took longer to achieve control and had a faster resurgence of activity. It is speculated that difference in temperature may have influenced IFA exposure to the chemical treatments. Thus, warm season replication of this trial is also scheduled for 2004 to examine potential differences over season.

Figure 1. Treatment efficacy in eliminating active IFA infestations when applied to single mound plots (7.07 ft²) – Mississippi 2003

Active Mounds

Weeks After Treatment

—✕— dry control   ⋯ wet control   —△— deltamethrin   ⋯ bifenthrin flowable   —□— bifenthrin granular
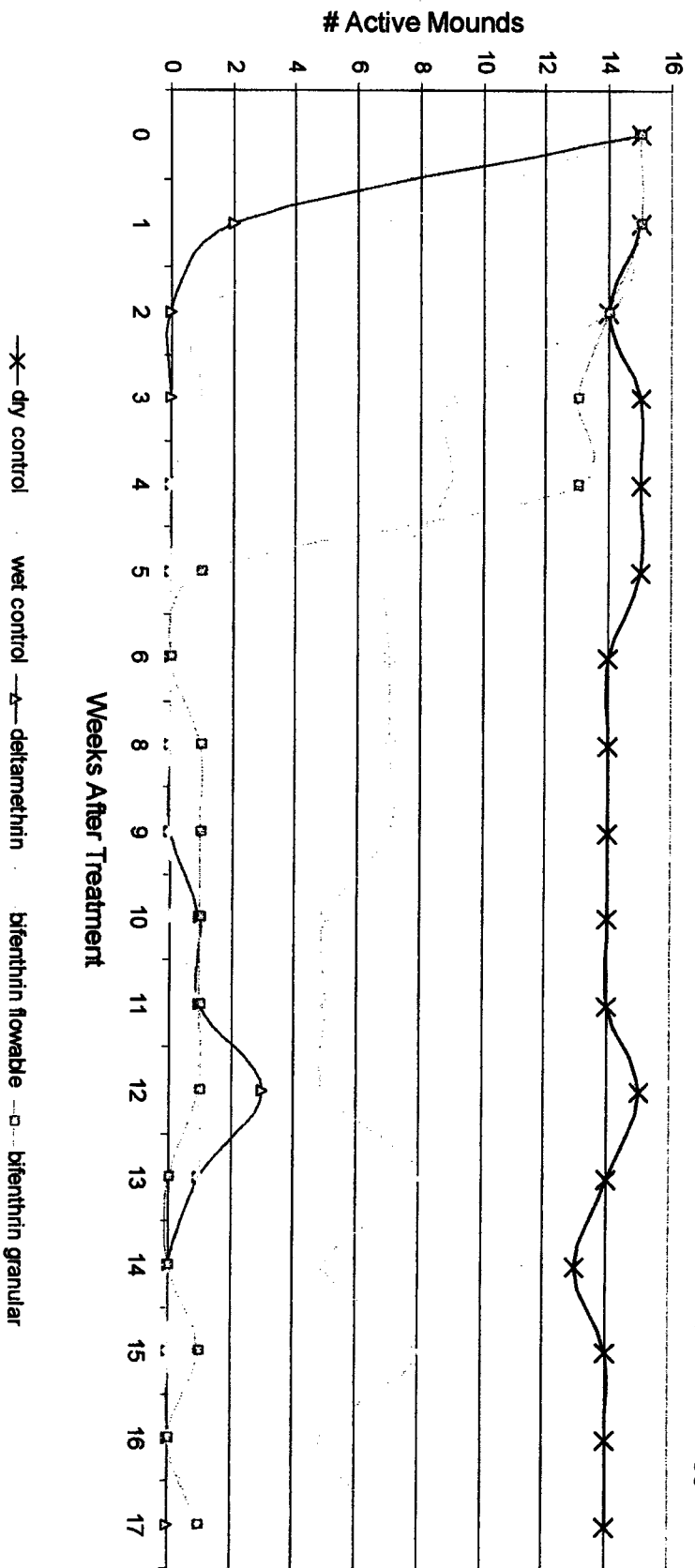
Figure 2. Treatment efficacy in eliminating active IFA infestations when applied to single mound plots (7.07 ft^2) – Mississippi 2003

— dry control   wet control   lambdacyhalothrin high rate   lambdacyhalothrin low rate   — carbaryl

Weeks After Treatment

Active Mounds
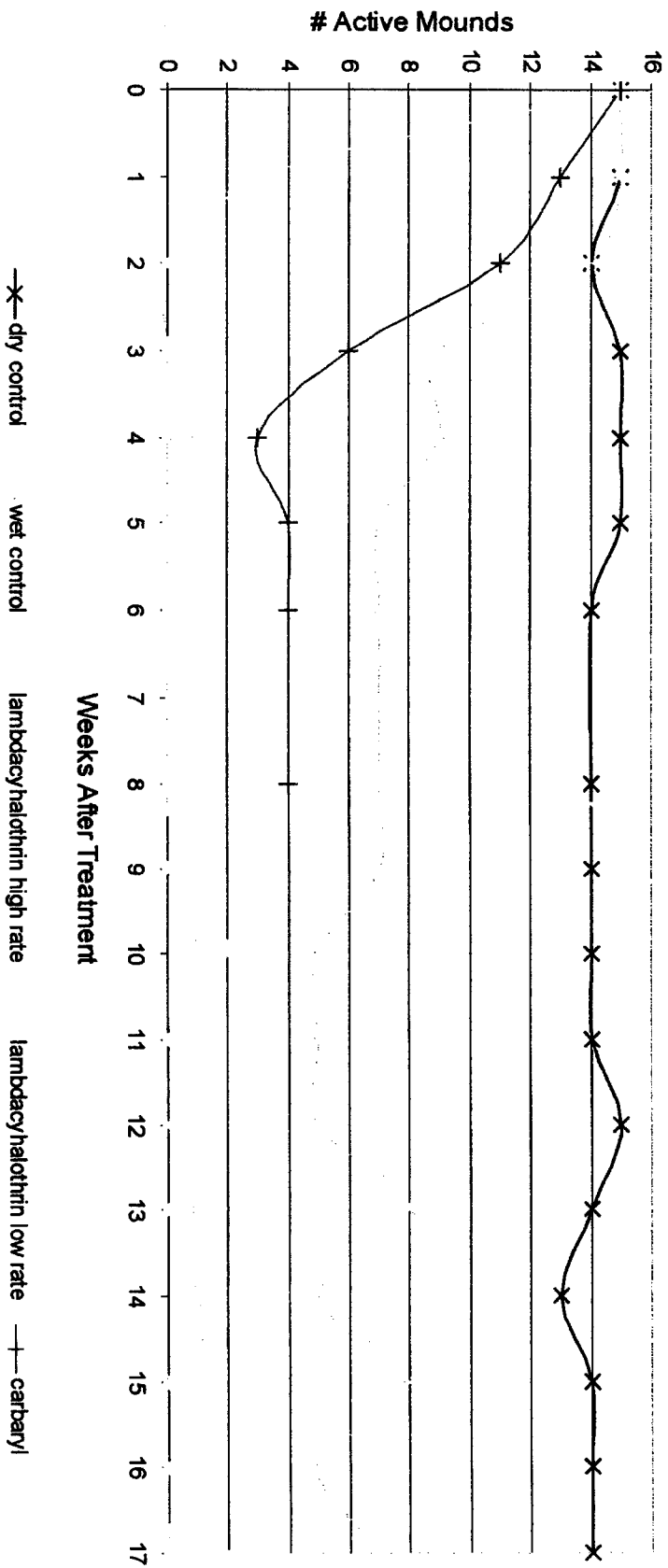
Figure 3. Weather measured at the Mississippi trial site through the duration of the test.
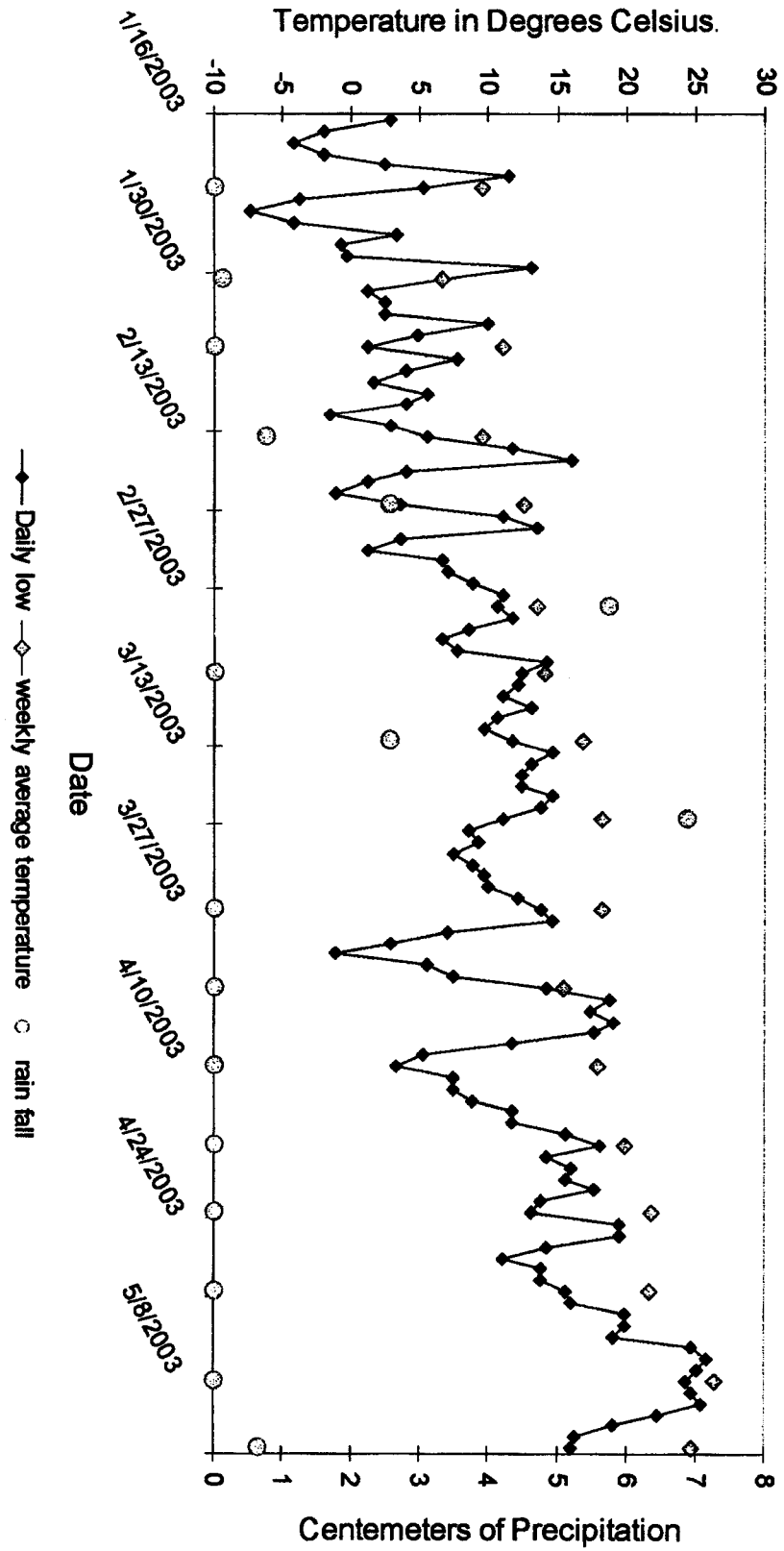
REFERENCES CITED

Brooker, J.R., R.A. Hinson, and S.C. Turner. 2000. Trade flows and marketing practices within the United States nursery industry: 1998. 2000 Southern Cooperative Series Bull.

Collins, H.L. and A-M. Callcott. 1995. Effectiveness of spot insecticide treatments on red imported fire ant control. J. Entomol. Sci. 30: 489-496.

Franke, O.F. 1983. Efficacy of tests of single mound treatments for control of red imported fire ants. Southwest. Entomol. 8: 42-45.

Hays, S.B., P.M. Horton, J.A. Bass and D. Stanley. 1982. Colony movement of imported fire ants. J. Georgia Entomol. Soc. 17: 266-272.

Williams, D.F. and C.S. Lofgren. 1983. Imported fire ant control: evaluation of several chemicals for individual mound treatments. J. Econ. Entomol. 76: 1201-1205.

PESTICIDES APPLIED TO INDIVIDUAL FIRE ANT MOUNDS DURING WINTER MONTHS AS POTENTIAL TREATMENTS FOR CERTIFYING NURSERY STOCK – 2003 TENNESSEE TRIAL

Jason B. Oliver[1], Shannon S. James[2], Anne-Marie Callcott[2], Nadeer N. Youssef[1], and Karen M. Vail[3].

[1] Tennessee State University, Institute for Agricultural and Environmental Research, Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110.
[2] USDA-APHIS-PPQ, Soil Inhabiting Pests Laboratory, 3505 25th Avenue, Gulfport, MS 39501.
[3] The University of Tennessee, Department of Entomology and Plant Pathology, 205 Ellington Plant Sciences Building, Knoxville, TN 37996-4560.

Introduction. The green industry (greenhouse and nursery crops) is an important component of the U.S. economy. Tennessee is currently ranked 6th in the nation in total nursery plant sales (USDA-NASS 2001). However, among the top six states in nursery sales, Tennessee has the highest percentage (33.9%) of total nursery sales in the field plant category (e.g., balled and burlapped [B&B]) (Brooker et al. 2000). Consequently, Tennessee nursery producers will be impacted more by fire ant regulations directed at field plant production than nursery producers in other states. Current certification treatments specified by the Federal Fire Ant Quarantine for field nursery plants are impractical, and include: 1) an in-field broadcast treatment of bait followed 3-5 days later by granular chlorpyrifos, 2) a B&B drench with chlorpyrifos, or 3) a B&B dip in chlorpyrifos. All of these treatments utilize chlorpyrifos. The broadcast field treatment is very expensive (~ $5001 treated hectare) due to the required chlorpyrifos formulation. Most producers presently opt for the daily drench treatments, because dip treatments require specialized equipment and disposal of large quantities of pesticide. In addition, dip treatments are hazardous, messy, labor intensive, disrupt root balls, and frequently cause plant phytotoxicity. However, daily drench treatments are also labor intensive, because root balls must be repeatedly treated and handled over a 4-day period (3 days treated/1 day re-entry interval). Both dip and drench treatments result in shipping delays and have environmental consequences from using large volumes of chemical in limited areas. Previous work at the USDA-APHIS Soil Inhabiting Pests Laboratory (SIPL), Gulfport, MS and in Tennessee has indicated some pesticide products have potential to satisfy field nursery plant certification requirements during winter shipping months (Oliver et al. 2002). The objectives of this study were to determine: 1) alternative pesticide treatments for field nursery stock that could be substituted for chlorpyrifos during the winter shipping season, 2) pesticide efficacy in a small treatment area, 3) the period of pesticide efficacy, and 4) if drench treatments applied at rates lower than labeled drench recommendations (i.e., broadcast rate) are effective at eliminating fire ant colonies. The study reported here was done in conjunction with a replicated trial performed by SIPL during winter 2003 (also being reported at this Conference).

Materials and Methods. Five pesticides were evaluated to assess their potential as individual field nursery plant treatments during the winter months in Tennessee. Fire ant mounds were treated at two sites during the study, including a commercial nursery in Franklin County, TN at 321 m elevation (15 mound replicates per treatment) and a residential development in Sequatchie County, TN at 634 m elevation (2 mound replicates per treatment) on February 5 and 6, 2003.

Based on previous test results, fire ants at these sites were likely *Solenopsis invicta* x *Solenopsis richteri* hybrids. Treatments were directly applied to individual fire ant mounds in a small treatment area (0.667 m²). Treatments included drenches of Sevin SL, DeltaGard GC 5SC, Talstar Nursery F, and Scimitar GC and a broadcast Talstar GC Granular treatment. These insecticides were applied at labeled broadcast rates for fire ant or subsurface insects (Turf and Ornamental Reference 2004). Broadcast rates typically are lower and require less water than pesticides applied as individual mound drenches for fire ants. However, liquid treatments used in this study were drench applied at the broadcast rate in 7.6 L water with at least 3.8 L applied directly to the mound. The Talstar GC Granular treatment was evenly broadcast over the treatment area using a saltshaker and was not irrigated after pesticide application. Control treatments included mounds that received 7.6 L water with at least 3.8 L applied directly to the mound and mounds that received no treatment.

Results. The Sevin SL and Talstar GC Granular treatments were ineffective at eliminating all fire ant colonies. The DeltaGard GC 5SC, Talstar Nursery F, and Scimitar GC treatments each provided from 4 to 9, 3 to 10, and 8 to 16 weeks post treatment control within the treated zone, respectively. Unfortunately, one colony at the Sequatchie County site in the Talstar Nursery F treatment required 8 weeks for complete elimination. Scimitar GC required longer to eliminate all fire ant colonies than Talstar Nursery F and DeltaGard GC 5SC, but also maintained the treated area ant-free for the entire 16 weeks of the trial. At the Franklin site, between the periods of March 21 and April 17, mound numbers in the irrigated and non-watered control treatments declined by 67% and 40%, respectively. At the Sequatchie site, all control treatment mounds were inactive by April 17. The reductions in control treatment mounds coincided with several periods of sharp temperature decline in late March and early April, which may explain the loss of activity seen in control treatments.

Discussion. The results of the Tennessee portion of this replicated trial indicate the three-pyrethroid drench treatments have potential as substitutes for the current chlorpyrifos regimens required by the Federal Fire Ant Quarantine to certify field nursery plants. Growers would need to wait at least one month before field plants could be harvested and shipped. Depending on the pesticide utilized, the post-harvest certification period would last 4 to 16 weeks before some form of re-treatment would be needed. The small treatment area (0.667 m²) was sufficient to maintain ant free areas with the pyrethroid drench treatments. In addition, the broadcast rates of the pyrethroid drench treatments were effective. The application of the drench treatments at broadcast rates will reduce treatment expense for nursery producers. Temperatures were primarily in the 5 to 20°C range during this trial. Therefore, the pyrethroid drench treatments were still effective despite relatively cool temperatures. At the Franklin nursery site, some mounds were adjacent to field nursery trees (≤ 5 cm caliper). However, pesticide efficacy was not reduced by the presence of a nursery plant. The soil at the Franklin site was a silty clay loam, while the soil at the Sequatchie site was a sandy clay loam. Products were effective despite high clay content soils.

Shepard], and FMC Corporation [Dr. John W. Long and Geri Cashion] for providing pesticide products used in this study.

References Cited

Brooker, J.R., R.A. Hinson, S.C. Turner. 2000. Trade flows and marketing practices within the United States nursery industry: 1998. 2000 Southern Cooperative Series Bull.

Oliver, J.B., D. Bailey, K.M. Vail, and A.-M. Callcott. 2002. Summary of insecticide tests directed at managing imported fire ants in field nurseries. Proc. 2002 Imported Fire Ant Conf., Athens, GA. pp. 152-158.

Turf and Ornamental Reference for Plant Protection Products. 2004. C&P Press. New York, NY.

USDA National Agricultural Statistics Service. 2001. Nursery sales reach $ 3.3 billion in 17- states survey. Tenn. Green Times. 2(4): 15 (Actual report: http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/).

Chemical Control

Advances in Imported Fire Ant Bait Technology:
Extinguish®R Plus, a blend of methoprene and hydramethylnon, and the modified Herd GT-77 Model Air-Assisted Applicator

Bastiaan "Bart" M. Drees, Texas A&M University, Doug Vangundy, Wellmark International, and David Herd, Herd Seeder Company

Two advances in fire ant bait technology have recently been achieved after years of developmental effort and are reported in this article. An accounting of the historical development of integrated pest management programs for the red imported fire ant, Solenopsis *invicta* Buren (I lymenoptera: Formicidae), can be found in Drees and Gold (2003). The development of the "Two-Step Method" for fire ant control began in 1992 and continues as new products are introduced to the market (Drees 2003a). The development of a fire ant bait formulation containing methoprene also began in 1992 with registration by the Environmental Protection Agency in 1998 (Williams et al. 2001; see 1992-1996 reports posted on http:Nfireant.tamu.edu). The dcsirc to make bait product applications to larger areas of land has resulted in the development of improved treatment methods such as the air-assisted applicator or "bait blower."

"Hopper blend" development. The concept of mixing an insect growth regulator (IGR) with a metablic inhibitor was initially suggested and practiced by pest control operators using a blend of fenoxycarb and hydramethylnon products, and was evaluated in early field trials (Drees, et al., 1995). The combination of a faster-acting metabolic inhibitor plus a slower-but-longer acting insect growth regulator (IGR) bait product such as Extinguish™, applied at half rates (0.75 lb) of each as a "hopper blend" (**Fig. 1**), has been demonstrated to provide a performance profile that is faster and longer lasting than either product applied individually at full rate (Drees, 2001). This concept led to a 24(c) registration by the Texas Department of Agriculture for BASF Corp. for the blending of hydramethylnon bait (Amdro® Pro or Siege® Pro) plus methoprene (Extinguish®) for 2002 and 2003. A supplemental label allowing for the use of the blend was issued by Wellmark International in 2003.
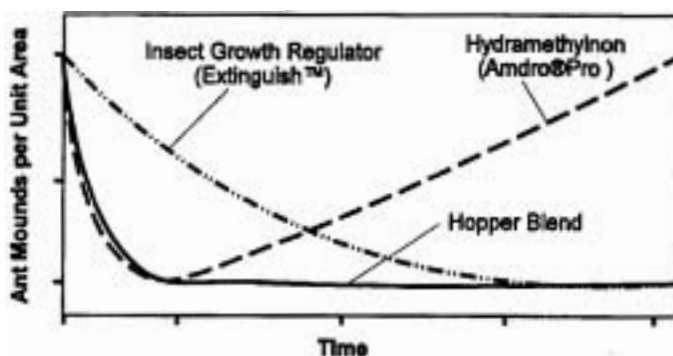
Fig. 1. Hypothetical performance profile illustration of the "hopper blend" (Drees 2001).

Hopper blend characteristics. The blend of hydramethylnon (Probait®R, Amdro®RPro, etc.) and s-methoprene (Extinguish™ or other IGR) baits, applied at reduced rate (50:50 blend at ½ rates of each to a unit area), has little effecting on the cost of treatment. Longer intervals between needed applications provided by the IGR component can reduce cost of treatment programs over time. In addition, the ability of ant colonies to share the IGR product resulting in "bleeding effects" of the treatment provides additional coverage of missed swaths (Drees et al. 1992).

Evaluations of hopper blend treatments have demonstrated effectiveness of this treatment combination (Barr et al., 2000 and 2003, Nester et al. 2003). This has become the standard treatment for use in the USDA-ARS Area-Wide fire ant program, in which large-scale use of the hopper blend provided roughly 90% control, ranging form <80 to 98% (**Table 1**) (Drees, 2003a).

Table 1. Results from "hopper blend" treatment used in the USDA-ARS Areawide Suppression of Fire Ants Program (Drees 2003a).

Location	Site	Size (acres)	Percent Control
Florida	Control	324	<80% (4 wks)
	Treatment	336	>93% (4 wks)
Mississippi	Control	253	98% (4 wks)
	Treatment	228	85% (4 wks)
	Treatment	253	90% (4 wks)
Oklahoma	Control	150	89.4% (8.6 wks)
	Treatment	150	96.7% (8.6 wks)
	Treatment	150	91% (8.6 wks)
South Carolina	Control	300	not reported
	Control	300	not reported
Texas	Control	300	90% (10 wks)
	Treatment	300	88% (10 wks)

In 2004, Wellmark International will be releasing Extinguish® Plus as a pre-blended product containing 0.365% hydramethylnon and 0.250% s-methoprene (Drees 2004a). The broadcast application rate for imported fire ants is 1.5 lbs. product per acre. Since no mixing is required, this product will be easier and more convenient to apply. It is registered for use in residential and commercial property, container or nursery stock, sod farms and commercial turf, landscape areas, golf courses, and other non-cropland areas such as airports, roadsides, cemeteries, commercial grounds, parks, kennels, school grounds, athletic fields, campgrounds, and other recreational

areas, as well as grounds surrounding poultry houses (excluding runs and ranges), or corrals and other animal holding areas (Drees 2004a).

Unfortunately, the new product is not registered for application to pasture and range land. However, this use is covered by both a Supplemental label from Wellmark International and it is now included on the "new" 2003 Amdro® Pro Fire Ant Bait label (see product label, http://www.cdms.net/manuf/1prod.asp?pd=4236&lc=2). Directions allow Amdro® Pro to be blended with Extinguish™ at a rate of 0.75 pound of each product applied at 1.5 lbs. of the blend per acre. In Texas and most other southeastern United States, this blend of products can be applied to non-agricultural lands and grass forage (pasture and rangeland).

Advances in bait application technology. From 1997 to 2001, Dr. Charles Coble, Agricultural Engineer at Texas A&M University, developed a prototype truck-mounted industrial ant bait applicator with support from the Texas Department of Transportation (TXDoT) and the Texas Imported Fire Ant Research & Management Project (**Fig. 2**). This device was designed to be capable of applying the recommended rate of ant bait (1 to 1.5 lbs. product per acre) to the side of the vehicle while traveling up to 30 miles per hour. Swath width varied with wind direction but ranged from a maximum of 30 to 50 feet. TXDoT had commissioned the development of the prototype as a possible method of rapidly treating roadsides, rest stops and right of ways for fire ant control (Drees and Frisbie 2002). Reports of development and demonstration of the prototype bait blower device at Texas A&M University can be found on the web site, http://fireant.tamu.edu/research2000-2002ResDemHbk.htm#blower.

Fig. 2. Prototype "bait blower" developed by Dr. C. Coble (Drees and Frisbie 2002).
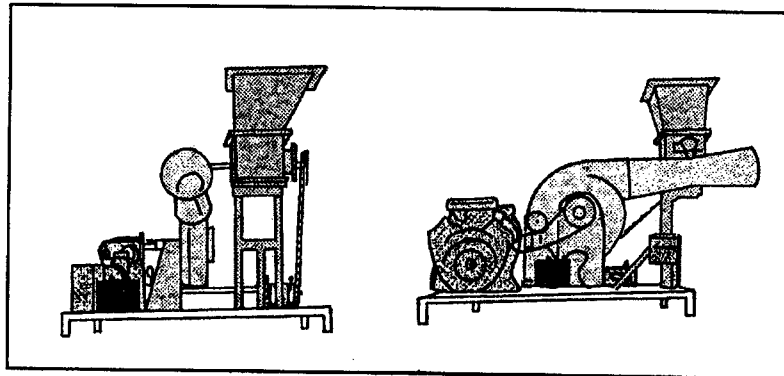
The prototype blower was demonstrated in the fall of 2000, when 50 acres of Magnolia Gardens Nursery in Montgomery County were treated within two hours. The nursery's blocks of containerized ornamental crops could be treated from the roadways through the nursery without applying bait to the roads themselves. It was also used to treat the Lago Santa Fe 80-acre subdivision in Galveston County in April 2002 (Nester et al. 2003). In addition to use for treating right of ways, commercial nurseries, and residential areas, this new modification may increase the likelihood of large-scale ant management by municipalities in fire ant abatement programs and other forms of community-wide fire ant management programs.

Commercial availability. Herd Seeder Company, Inc. (P.O. Box 448, Logansport, Indiana

46947-0448; 219/753-6311 info@herdseeder.com), has developed and demonstrated a new air-assisted version of the only available vehicle-mounted ground applicator suitable for broadcast-applying imported fire ant bait products, the GT-77 model Herd Seeder. Substantial modifications have been made to the original design: it has been converted to an air-assisted directional applicator or a "bait blower." This new product line will become available within the coming year directly from the company (Drees 2004b).

Fig. 3. GT-77 model Herd Seeder modified for air-assisted fire ant bait product application (Drees 2004a).

This new development is a combination of an Echo® leaf blower, that employs a small gasoline-powered engine and a conventional GT-77 model Herd Seeder in such a way that the airstream generated by the leaf blower is directed into a directional shoot. The blowers produces an airstream of 150 miles per hour. The metering plate supplied with the seeder has holes that are pre-calibrated to allow the vehicle driver to apply the right amount of bait product being used. The seeder and blower are both affixed to a swivel mounting structure that allows the user to direct application to either side of the vehicle to avoid application to roadways. It can apply the correct rate of product traveling up to 20 miles per hour for large-scale treatments.

By dismantling the component parts, the user can continue to benefit from traditional uses of both the seeder and the blower. In addition, people who already own a GT-77 model seeder only need to purchase additional component parts to convert to the bait blower.

The GT-77 model seeder, used alone, can apply the recommended amount of ant bait product per acre at a moderate 7 to 10 mile an hour speed. It produces a 20 ft. wide swath from the center, which is ideal for applications to pastureland, sod farms, golf courses, large landscapes and park land. However, when applying from roadways or "cuts" in containerized nursery operations, this method would apply a lot of bait material to the roadways. The new design dramatically increases rate of application and swath width, thereby reducing treatment time necessary to treat larger areas.

Literature cited:

Barr, C. L., R. L. Best, and B. M. Drees. 2000. A field comparison of five broadcast baits as full rate, hopper blend and skip-swath applications. Proceedings 2000 Imported Fire Ant Conference, Chatanooga, TN, April 6-7. pp. 84-89.

Barr, C. L., R. L. Best, R. Jahn, B. Gregson, and D. Fromme. 2003. Different ratios of s-methoprene and hydramethylnon baits as hopper blends for the suppression of red imported fire ants. Red Imported Fire Ant Research and Management Program Result Demonstration Handbook 1999-2003 on http://fireant.tamu.edu/research. Texas Cooperative Extension, Texas A&M University System, College Station, TX.

Drees, B. M. 2001. 24(c) Registration for Texas: "Hopper Blend" of Hydramethylnon Plus Methoprene. Fire Ant Trails 5(2):1-4 on http://fireant.tamu.edu Texas Cooperative Extension, Texas A&M University System, College Station, TX.

Drees, B. M. 2003a. Areawide pest management partnership initiative: Suppression of fire ants. Fire Ant Trails 6(2):1-4 on http://fireant.tamu.edu Texas Cooperative Extension, Texas A&M University System, College Station, TX.

Drees, B. M. 2003b (rev.). An organic two-step method for imported fire ant control. Fire Ant Plan Fact Sheet FAPFS037 on http://fireant.tamu.edu Texas Cooperative Extension, Texas A&M University System, College Station, TX, 5 pp.

Drees, B. M., C. L. Barr, M. E. Heimer, and R. Leps. 1995. Reducing treatment costs for fire ant suppression in Texas cattle production systems. in Proceedings of the Fifth International Pest Ant Symposia and the 1995 Annual Imported Fire Ant Conference (ed. S. B. Vinson and B. M. Drees), San Antonio, Texas. pp .146-154.

Drees, B. M. 2004a. Extinguish® Plus Released for Spring 2004; Amdro® Pro "Hopper Blend" Instructions Provided for Use in Pasture and Range Lands. Fire Ant Update 1(3):1-4 on http://fireant.tamu.edu Texas Cooperative Extension, Texas A&M University System, College Station, TX.

Drees, B. M. 2004b. Herd Seeder Company Introduces Air-Assisted Modification of GT-77 Model Seeder for Applying Fire Ant Bait Products. Fire Ant Update 1(3):1-4 on http://fireant.tamu.edu Texas Cooperative Extension, Texas A&M University System, College Station, TX.

Drees, B. M., C. L. Barr, and S. B. Vinson. 1992. Effects of spot treatments of Logic® (fenoxycarb) on polygynous red imported fire ants: an indication of resource sharing? Southwestern Entomol. 17(4):313-319.

Drees, B. M., and R. E. Frisbie. 2002. Overview of the Texas Imported Fire Ant Research and Management Project (B. M. Drees, ed.). Southwestern Entomologist Supplement No. 25:1-6.

Drees, B. M., and R. E. Gold. 2003. Development of integrated pest management programs for the red imported fire ant (Hymenoptera: Formicidae). J. Entomol. Sci. 38(2):170-180.

Nester, P. R., C. P. Bowen, and B. M. Drees. 2003. The Lago Santa Fe Fire Ant Project: Community-wide Imported Fire Ant Management in Texas in Proceedings of the Red Imported Fire Ant Conference (L. Greenberg and C. Lerner, eds.), Palm Springs, CA, March 30-April 1.

Williams, D. F., H. L. Collins and D. H. Oi. 2001. The red imported fire ant (Hymenoptera: Formicidae): an historical perspective of treatment programs and the development of chemical baits for control. Amer. Entomol. 47: 146-159.

How Fast is Fast?: Indoxacarb Broadcast Bait

Charles L. Barr, Texas Cooperative Extension

In 2003, two test were conducted to determine the effectiveness of the active ingredient indoxacarb, manufactured by DuPont, formulated on a conventional broadcast bait for the control of red imported fire ants (Solenopsis invicta Buren). The product, tested at three rates, was found to eliminate fire ant mound within a week and suppress foraging within a few days.(Barr, in press) This trial was designed to test the commercial formulation of the product, 0.045% indoxacarb, and determine just how fast mound and foraging were eliminated by conducting repeated, short-interval evaluations immediately after bait application.

Materials and methods

The test was conducted at the Fayette County Regional Airport near the town of LaGrange in central Texas. The airport consists of a 5,000 foot runway with a taxiway over about three-quarters of its length. Soil was a sandy loam over clay. At the time of test establishment, the area had been under a prolonged period of dry conditions. Fire ant mounds were generally small and often had to be dug with a shovel to check for ant activity, particularly during the heat of the day.

This "airport method" (Barr, in press) of plot design used 0.25-acre plots beginning 10 feet either side of runway lights (200 feet apart) and extended in a strip adjacent to the pavement and out 60.5 feet. The sample area consisted of a strip beginning and ending 10 feet inside the treatment area, 20-feet wide and adjacent to the pavement for a sample area of 3,200 ft.^{2}.

Plots were pre-counted on June 30, 2003. Plots were arrayed from highest to lowest pre-count, divided into four equal groups (replications) and treatments assigned within replications so that the total number of active mounds per treatment for all four replications was as equal as possible. (Barr and Best, 2002). Mound evaluations were conducted using the minimal disturbance technique with a pointed tool handle or small shovel, depending on soil moisture.

To assess fire ant foraging, four, 118th-inch thick slices of hot dog wieners were skewered to the ground using wire surveyor's flags in each plot. The hot dogs were placed by walking 23 paces from a runway light then placing a slice. The remaining three slices were placed at 10-pace intervals, which left 23 paces to the next runway light. This spacing put all the slices equidistant from the nearest untreated plot edge. Slices were placed in the shade of vegetation to encourage ant foraging in full sun. All slices were put out in one lap of the runway, then immediately retrieved in the same order, giving an exposure time of about 40 minutes. The number of ants on a slice were estimated and recorded as 0, 5, 10, 25, 50 or 100.

Treatments included: indoxacarb, 0.045% at 1.5lbs./acre; Amdro® Fire Ant Bait (0.73%) at 1.5 lbs./acre; Ortho® Fire Ant Bait (0.015% spinosad) at 4 lbs./acre; Firestar® bait (0.00015% fipronil) at 1.5 lbs./acre; Talstar 2G (0.2% bifenthrin) at 25 lbs./acre and an untreated control. All treatments were replicated four times.

Bait treatments were applied from 7:30 - 8:45 p.m. on June 30, 2003. Pre-weighed packets of bait were placed at the beginning of each plot prior to application so that they could be applied sequentially along the length of the runway. Applications to the four bifenthrin (Talstar 2G) plots began at 8:45 p.m. and ended at about 9:15 p.m. All applications were made using an EarthWay® Ev-N-Spred hand-held "belly bumper" spreader.

Evaluations were conducted July 1-3, 7, 22, 31; August 11; September 1; October 1 and November 1. To avoid the heat and resulting suppression of mound and foraging activity, the July - September evaluations were begun between 7:30 and 8:00 a.m., concluding 11:00 to noon. Thus, the first three evaluations were conducted at 12, 36 and 60 hours. Data were analyzed using PC SAS ANOVA, means separated using Tukey's studentized range (HSD) test, $P < 0.05$.

Results and Discussion

As shown in Table 1 and Figure 1, indoxacarb treatments resulted in a steady, rapid reduction in mound activity until, at 60 hours post-treatment, it had achieved 98% control. There was, in fact, only one active mound in all four plots. Hydramethylnon (Amdro) and bifenthrin (Talstar) reached 86% control, 2.0 mounds per plot, within seven days. This speed of control is unusually fast for hydramethylnon, which usually takes two to four weeks to reach maximum suppression. Bifenthrin granules (Talstar 2G) on the other hand, can work faster, but requires either rainfall or irrigation. The rapid drop by day seven was likely the result of a major rain event on July 5 and 6.

Foraging suppression by indoxacarb was even faster. As shown in Table 2 and Figure 2, foraging dropped by 85% within 12 *hours* of application - literally overnight - and continued to drop to zero within 60 hours. As with the mound counts, hydramethylnon and bifenthrin reached maximum suppression within seven days. Whereas seven-day maximum suppression of mound activity was quite fast for hydramethylnon, seven-day foraging suppression has been documented for this product (Barr, 2003). As before, rainfall likely activated bifenthrin.

Spinosad bait (Ortho) and fipronil bait (Firestar) performed uncharacteristically poorly in this test. The fipronil material, which was used in other tests with similar results, was sent to the manufacturer for analysis and was found to have a sub-standard level of active ingredient. Therefore, these results should not be considered typical for Firestar.

The spinosad bait, purchased from the Wal-Mart in LaGrange immediately prior to application, gave disappointing results. Maximum mound suppression was only about 65% and it yielded an unusual and unexpected foraging performance profile. It actually suppressed foraging faster and to a greater degree than indoxacarb, reaching 96% control within 12 hours, but foraging rebounded to less than 50% control in a week and continued to increase. It was as if the active ingredient's effects were transitory, something we have not encountered in previous work with this product.

In summary, indoxacarb bait proved to be the fastest-acting bait we have ever tested. Not only is it fast, but it is quite efficacious. Its speed of foraging suppression rivals that of contact insecticides and it does not require irrigation. It also kills the colonies below ground, which surface applications with contact insecticides may not do. Consumer and professional acceptance of fire ant baits has been very reluctant, due largely to their slow activity. Indoxacarb appears to overcome this problem and also provides very thorough, environmentally friendly control.

Literature Cited

Barr, CL. In press. Fire ant mound and foraging suppression by indoxacarb bait. J. of Ag. and Urban Ento..

Barr, CL. 2003. Speed of Red Imported Fire Ant Foraging Suppression Using Spinosad Broadcast Bait. RIFA Res.Result Dem Hdbk.1999-2003. Tx. Coop. Ext., Bryan, TX 77806

Barr, CL and RL Best. 2002. Product evaluations, field research and new products resulting from applied research. SW Ento. Supplement 25:47-52

Table 1. Mean number of active mounds. LaGrange Airport. Treated June 30, 2003.

Treatment	Mean number of active mounds per sample area, 3200 ft², 4 replications						
	Pre	12 hr.	36 hr.	60 hr.	7 day	21 day	30 day
untreated	14.75 a	14.75 a	13.25 a	12.00 a	15.75 a	16.50 a	16.50 a
indox.	14.25 .	11.25 .	4.50 b	0.25 c	0.00 b	2.25 b	2.25 b
hydrameth.	14.25 .	11.00 .	7.75 ab	5.25 bc	0.75 b	1.50 b	1.50 b
fipronil	14.50 .	14.00 .	11.75 ab	9.75 ab	8.75 ab	6.25 ab	6.25 ab
spinosad	14.50 .	13.50 .	8.25 ab	7.00 ab	7.00 ab	9.50 ab	9.50 ab
bifenthrin	14.25 .	13.25 .	7.25 ab	5.75 bc	0.00 b	2.25 b	2.25 b
F	0.03*	13.88	4.58	7.94	6.37	6.73	3.91
P	0.0001	0.0001	0.0055	0.0003	0.0011	0.0008	0.0110
R^2	0.3044	0.8810	0.7100	0.8089	0.7725	0.7821	0.6761
MSD	5.1085	5.3864	7.3042	5.7714	9.7499	9.4125	11.427

Means in the same column with different letters are significantly different ($P < 0.05$) using SAS ANOVA with means separated by Tukey's studentized range (HSD) test. df = 15. Model = treat and rep. *Treatment effects only, df = 5.

Table 2. Mean number of ants per hot dog slice. LaGrange Airport. Treated June 30, 2003.

Treatment	Mean number of ants per hot dog slice, 4 slices per plot, 4 replications					
	12 hr.	36 hr.	60 hr.	7 day	21 day	30 day
untreated	100.00 a	100.00 a	100.00 a	88.13 a	95.31 a	93.75 ab
indox.	13.75 c	0.625 d	0.00 d	7.81 b	25.00 c	38.13 c
hydrameth.	70.94 ab	58.75 c	39.06 bc	9.38 b	54.69 bc	59.38 bc
fipronil	93.75 a	93.75 ab	81.25 a	81.25 a	68.75 ab	100.00 a
spinosad	4.38 c	25.625 d	22.50 cd	56.88 a	85.94 ab	100.00 a
bifenthrin	54.69 b	67.188 bc	67.81 ab	0.00 b	19.38 c	40.63 c
F	19.49	23.49	17.10	16.57	9.05	7.03
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
R^2	0.6419	0.6836	0.6112	0.6038	0.4542	0.3926
MSD	30.776	28.447	32.244	31.979	36.569	36.293

Means in the same column with different letters are significantly different ($P < 0.05$) using SAS ANOVA with means separated by Tukey's studentized range (HSD) test. df = 87. Model = treat and rep. All plots had 100+ ants per hot dog slice during the pre-treatment evaluation. *Treatment effects only, df = 5.
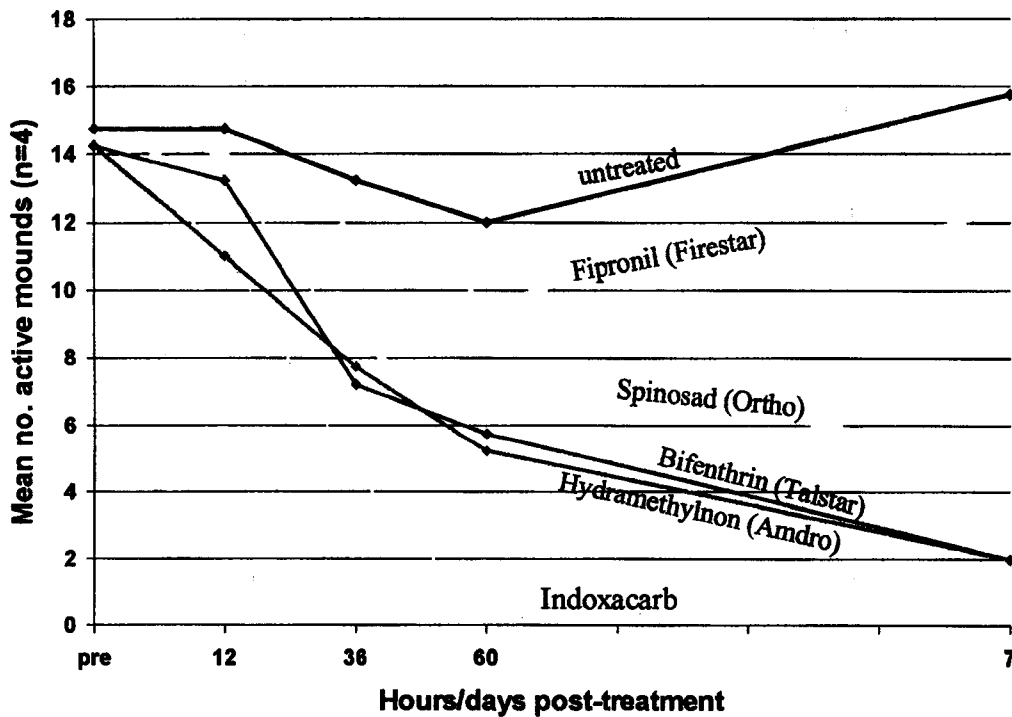
Figure 1. Mean number of active mounds, 4 replications, through 7 days post-treatment
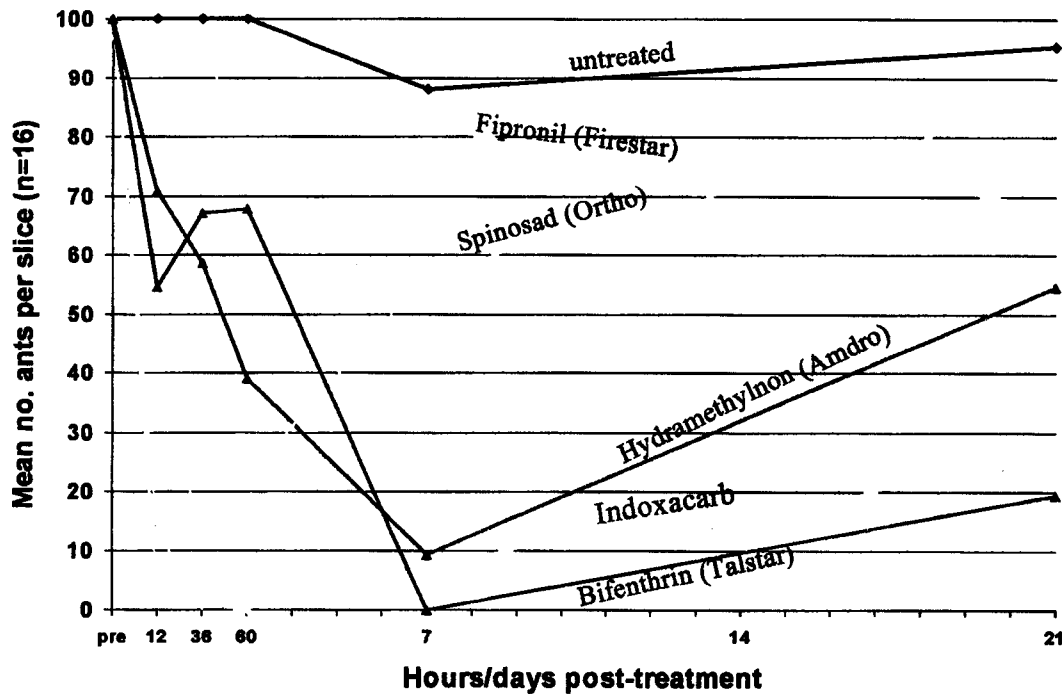
Figure 2. Mean number of ants per hot dog slice, 4 replications, 4 samples per replication, through 21 days post-treatment.

Lago Santa Fe Fire Ant Project, Santa Fe, TX: The Never Ending Story!

Paul R. Nester, Corrie P. Bowen and Bastiaan M. Drees
Extension Agent - IPM, County Extension Agent – AG/NS – Galveston Co.,
Extension Specialist – Entomology, respectively
Texas Cooperative Extension

Abstract

Managing the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) has been demonstrated to dramatically reduce the cost insecticide use, maintain control of fire ants and eliminate problems caused by the ant. This demonstration, conducted in the Lago Santa Fe community in Galveston County, Texas, demonstrated several recent advances in conducting community-wide programs, including: 1) the effectiveness of the "hopper blend" treatment (50:50 hydramethylnon plus s-methoprene ant bait); 2) application methods such as the truck-mountable industrial "bait blower"; and 3) scheduling treatments to reach a goal of maximum control for athletic events being hosted by the Lago Santa Fe Community, i.e., the 2002 and 2003 National Water Ski Championships, and U.S. Open Water Ski Championships. Fire Ant mound activity counts showed that after a single spring 2002 hopper blend treatment, fire ant activity was reduced 85% in the community of Lago Santa Fe before the scheduled 2002 water ski events. After an additional fall 2002 and spring 2003 hopper blend treatment, fire ant mound activity was down 95%, before the scheduled 2003 water ski events.

LABORATORY EVALUATIONS OF LIQUID TOXICANTS AGAINST RED IMPORTED FIRE ANTS

Beverly A. Wiltz, Daniel R. Suiter, and Wayne A. Gardner

University of Georgia, Department of Entomology, Griffin, GA

Introduction

We evaluated fipronil (Termidor SC), chlorfenapyr (Phantom), and bifenthrin (Talstar Flowable) for properties that contribute to their efficacy against the red imported fire ant. Laboratory assays were conducted to determine topical toxicity, mobility impairment, horizontal activity, and effectiveness as a barrier. A combination of characteristics determine which product is most effective for a particular application. Slow-acting, transferable insecticides maximize the effect to untreated individuals, while fast-acting or repellent insecticides are more effective as barrier treatments.

Materials and Methods

Experiment 1. Toxicity from topical treatments. Three treatments were tested for their ability to kill worker ants via a topical treatment: 0.5% chlorfenapyr, 0.06% bifenthrin, and 0.06% fipronil. Control ants were treated with water. Plastic boxes were prepared for treating ants by coating the inside walls with fluon to prevent escape and placing a piece of absorbent paper on the bottom of the box. Ten groups of 20 workers were poured onto the paper, directly sprayed with one of the suspensions and immediately transferred to a dry plastic box. Ant mortality in each replicate was determined at half-hour intervals for 6 hours.

Experiment 2. Mobility of topically-treated ants. Ten groups of approximately 100 workers were topically treated with chlorfenapyr, fipronil, bifenthrin, or water. From each group, 10 ants were placed into each of four 10 oz. portion cups. At half-hour intervals for two hours one cup from each group was inverted onto the center of a 14-cm diameter circle drawn on a piece of paper. Ants remaining inside the circles after 2 minutes were counted.

Experiment 3. Horizontal effects from dead ants. Fire ants were treated by dipping a ball of ants into one of the insecticides. Spraying does not work well for large groups of fire ants because the ants form a ball, shielding the interior ants from the insecticide. After treatment, ants were transferred to dry containers to die. Corpses were placed on plastic disks in fluon-lined plastic boxes containing untreated nest-mates. Because ants readily carry corpses to and from the nest site (Wilson et al. 1958, Gordon 1983), this method allows contact between treated and untreated individuals while minimizing contamination of the arena floor. Ants were provided a nest cell (Petri dish spray-painted black and half filled with dental Castone, with holes drilled through the dish at the Castone surface level to allow ants to move in and out), sugar water, and water. Each box contained a total of 300 ants, with 5, 10, or 20% treated. Controls received 60 ants killed by freezing. Boxes were covered and incubated at 10°, 20°, or 30°C for 3 days before determining mortality of untreated ants. The test was replicated six times for each chemical-temperature combination.

Experiment 4. Barrier tests. To determine if the insecticides would deter movement across a treated area choice tests were conducted using three boxes arranged linearly: a center chamber connected by paper bridges to two feeding chambers. The boxes on each side contained a nest cell, a sugar-water-soaked cotton ball, and a water-soaked cotton ball. To prevent a moisture differential that might influence ant movement, all nest cells were prepared by oven drying for 24 h at 50° C, cooling at room temperature and humidity for 2h, then adding 6 ml water.

Bridges were 15" x 2" strips cut from file folders, with the center 4" x 2" section treated with one of the insecticides or water (Table 1). Cinnamaldehyde (Cinnamite) was used as a positive (repellent) control. In preliminary tests, 0.6 ml of 1.5% cinnamaldehyde provided close to 100% repellency. The chemical was pipetted onto the paper, brushed over the desired area, and air dried for 1 hour. The paper was then turned over and the reverse side treated in the same way. The center section of the bridge to the second (control) feeding chamber was painted with water.

Ants were starved for 2 days with access to only water, then 300 were placed in the center chamber with no food or water. The number of dead and live ants in each of the 3 chambers was recorded after 24 hours. There were ten replicates for each treatment.

Table 1. Treatments applied to bridges for repellency tests

Chemical	Label rate	Bridge treatment (8 in^2)
	6gal 0.05% AI / 1,000 ft^2	1.2 ml 0.05% F5025
Bifenthrin	5 gal 0.06% AI / 1,000 ft^2	1.0 ml 0.06% bifenthrin
Cinnamaldehyde	N/A	0.6 ml 1.5% cinnamaldehyde
Chlorfenapyr	0.5 % AI for spot treatments	0.3 ml 0.5% chlorfenapyr
Fipronil	1.5 gal 0.06% AI / 1,000 ft^2	0.3 ml 0.06% fipronil
Water (control)	N/A	0.3 ml water

Results and Discussion

Experiment 1. Toxicity from topical treatments. Treatment with bifenthrin resulted in 100% mortality after 2.5 hours. After 6 hours, chlorfenapyr mortality had reached 83% and fipronil mortality had reached 35% (Figure 1).

Experiment 2. Mobility of topically-treated ants. For a chemical to be transferred among ants in the field, it would be important that sprayed ants remain alive and mobile for a period of time to enable interaction with untreated ants. Two hours after topical treatment, 50% of chlorfenapyr-treated ants and 85% of fipronil-treated ants remained mobile. All bifenthrin–treated ants were knocked down within the first 30 minutes (Figure 2).

Experiment 3. Horizontal effects from dead ants. For bifenthrin, mortalities were highest at 20°C, followed by 30°C and 10°C. At temperatures with higher mortalities, increasing the percentage of ants treated had a larger effect (Figure 3A). For chlorfenapyr, the 20% treatment had higher mortality than 5%, but neither differed from10%. Although temperature was not significant, elevated mortality was seen at 30°C when 10% or 20% of the ants were treated (Figure 3B). For fipronil, both temperature and percentage treated were significant. There was higher mortality at 20°C and 30°C than at 10°C (Figure 3C).
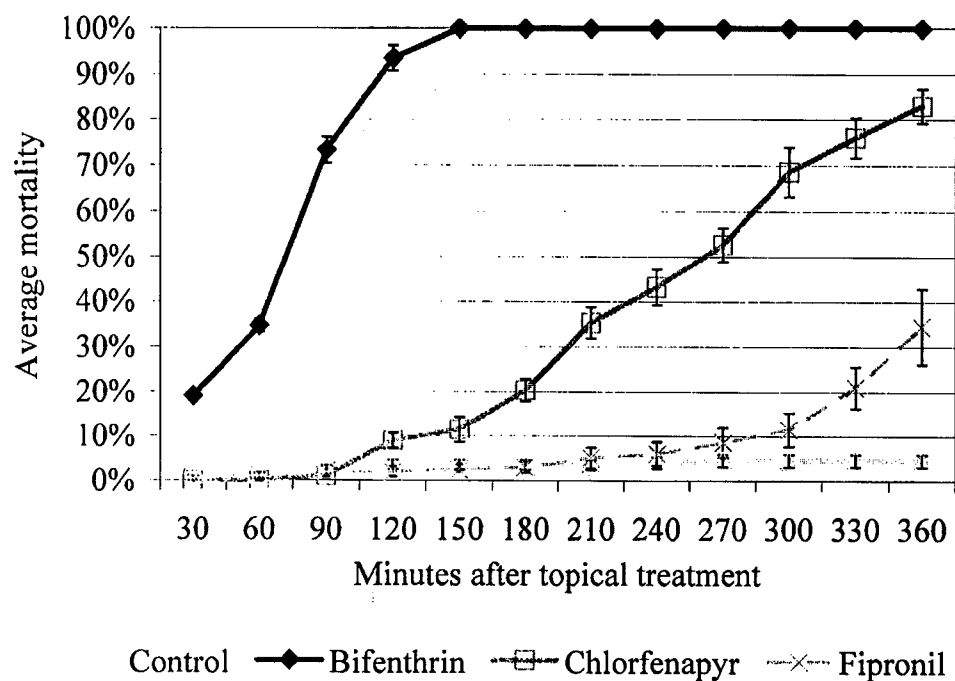
Figure 1. Mortality (mean ± SE) after topical treatment.
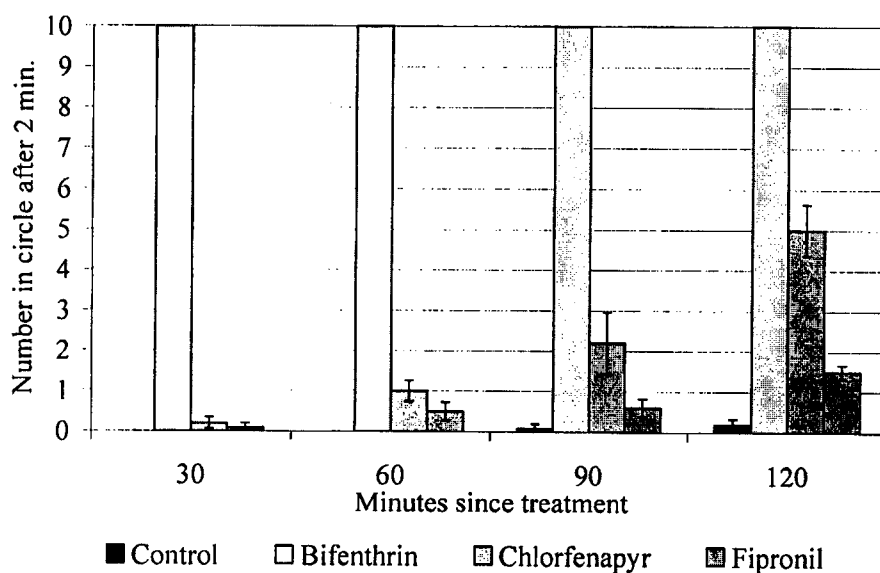
Figure 2. Mobility impairment after topical treatment. Bars represent the number (mean ± SE) of ants out of ten remaining inside circles after 2 minutes.

Experiment 4. Barrier tests. After 24 hours, the bifenthrin treatment and the positive control, cinnamaldehyde, had fewer ants than the water side. Among ants that had crossed the bifenthrin-treated bridges, there was 94% mortality. Lack of recruitment may account for the low numbers on the bifenthrin side relative to the control side.
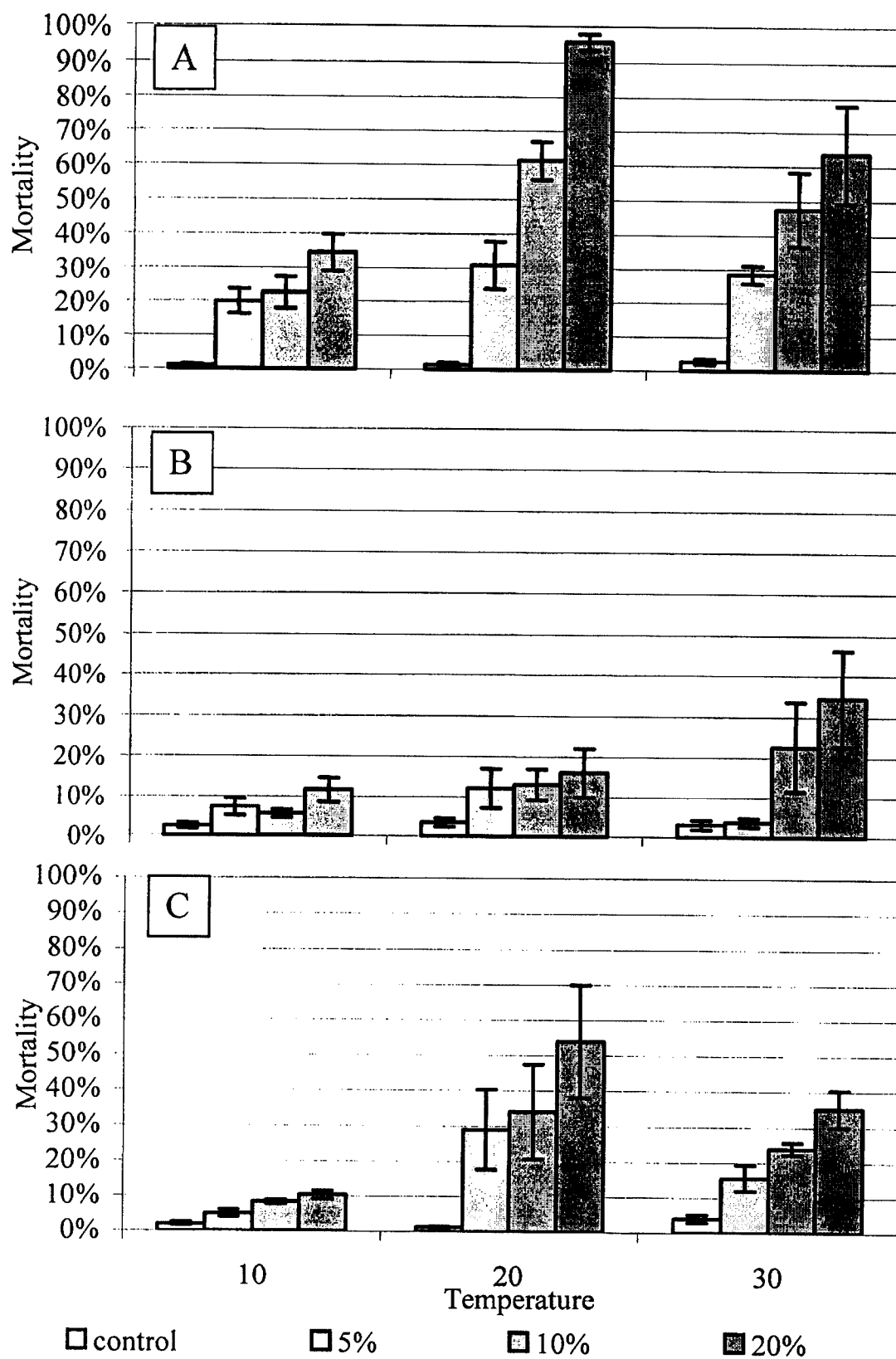
Figure 3. Mortality (mean ± SE) of untreated ants exposed to corpses of A) bifenthrin, B) chlorfenapyr, or C) fipronil-treated nestmates.

Many repellency tests measure the response of individual ants or the number of ants crossing a barrier over a short period of time (Vander Meer et al. 1988, Knight and Rust1990). This test differs in that it measures a group response rather than the movement of individual ants. By counting the number of ants remaining in each feeding chamber rather than the number crossing the barriers, we incorporate repellency and recruitment effects. Since there were no food or nests in the center chambers, ants successfully crossing bridges should have recruit more foragers to that side unless they were killed or immobilized quickly. Although the bifenthrin treatment had fewer ants than controls, results are not inconsistent with other studies that showed bifenthrin to be non-repellent (Oi and Williams 1996, Richman and Hooper-Bùi 2003). Our findings of 100% knockdown within 30 minutes and 100% mortality within 2.5 hours explain the lack of recruitment across bifenthrin-treated bridges.
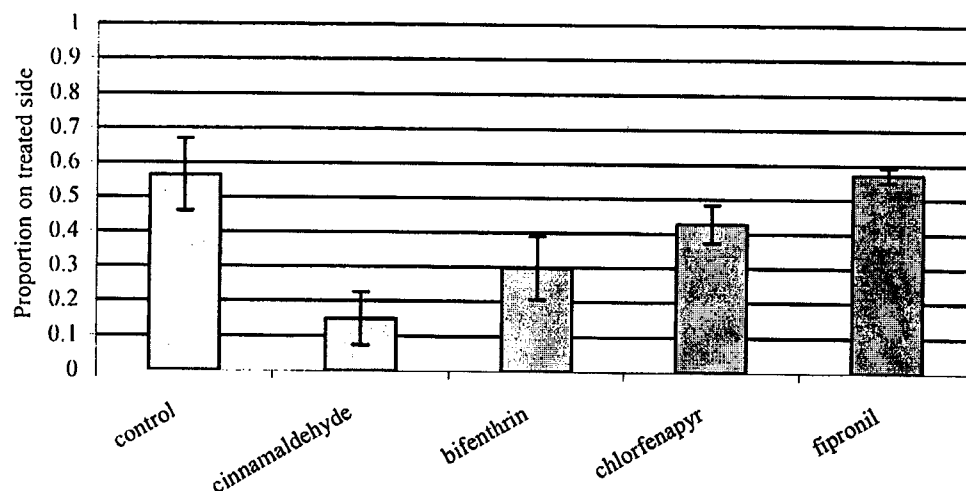
Figure 4. Proportion of ants on insecticide treated side in choice test.

References Cited

Gordon, D. M. (1983). "Dependence of necrophoric response to oleic acid on social context in the ant, Pogonomyrmex badius." J. Chem. Ecol. 9: 105-111.

Knight, R. L. and M. K. Rust (1990). "Repellency and efficacy of insecticides against foraging workers in laboratory colonies of Argentine ants (Hymenoptera: Formicidae)." J. Econ. Entomol. 83: 1402-1408.

Oi, D. H. and D. F. Williams (1996). "Toxicity and repellency of potting soil treated with bifenthrin and tefluthrin to red imported fire ants (Hymenoptera: Formicidae)." J. Econ. Entomol. 89(6): 1526-1530.

Pranschke, A. M., L. M. Hooper-Bùi and B. Moser (2003). "Efficacy of bifenthrin treatment zones against red imported fire ant." J. Econ. Entomol. 96(1): 98-105.

Richman, D. L. and L. M. Hooper-Bùi (2003). Mystery Solved? PCT. 31: 84-87.

Vander Meer, R. K., F. Alvarez and C. S. Lofgren (1988). " Isolation of the trail recruitment pheromone of Solenopsis invicta." J. Chem. Ecol. 14: 825-838.

Wilson, E. O., N. I. Durlach and L. M. Roth (1958). "Chemical releasers of necrophoric behavior in ants." Psyche 65(4): 108-114.

Biology & Ecology

Stimulation of Black Imported Fire Ants *(Solenopsis richteri* Forel) using substrate-borne vibrations and the effects on the parasitism of host ants by attacking phorid flies *(Pseudacteon curvatus* Borgmeier)

Esther Mwangi[1*], Roger Hasse[2], Paul Lago[1], Richard Buchholz[1], and Douglas Streett[3]

[1] *Department of Biology, University of Mississippi, University, MS 38677*
[2] *National Center for Physical Acoustics, University of Mississippi, University, MS 38677*
[3] *USDA-A RS-BCMRRU, Mississippi State, MS 38766*

INTRODUCTION

The Black Imported Fire Ant *(Solenopsis richteri* Forel) is an invasive insect species that has negatively affected regional ecosystems in the southern and southwestern United States (1). The United States Department of Agriculture, Agricultural Research Service (USDA-ARS), is currently addressing the problem of fire ant expansion by using an integrated approach involving chemical and biological control methods. In collaboration with the USDA-ARS, the National Center for Physical Acoustics (NCPA) at the University of Mississippi is investigating whether acoustics plays a role in both intraspecific communication and reproduction of BIFA. It is known that ants do not possess tympanal organs used to sense airborne sound (2), however a recent paper suggests that fire ants are sensitive to airborne sound in the near-field (3) and use stridulation as a form of communication. This work is a multi-part study focused on the application of substrate-borne vibrations and understanding how the behavioral responses of fire ants are affected in the process, under tethered and non-tethered conditions. Part I of the research-involved stimulation of individual black imported fire ants and the subsequent visual observations of their leg movements in response to the applied stimuli. The frequencies and corresponding substrate displacements that major fire ant workers responded to were then applied to colonies of fire ants in experimental arenas and the behavioral responses observed. Finally, Part III of the study involved the introduction of substrate-borne vibrations to a tub containing fire ants in the presence of attacking phorid flies *(Pseudacteon curvatus* Borgmeier) at the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU) in Mississippi State, Mississippi. The effects of vibrational stimulation on the parasitism of host fire ants were determined and then compared with the results obtained for both a control tub (no stimulation) and a tub, which employed an inverted-cup system to induce active trailing behavior in the animals.

PART 1: MATERIALS AND METHODS

A wooden rod 40.6 cm long and 1.27 cm in diameter was secured in a fixed-free configuration using a vise, and the top surface of the rod focused under a microscope with 25× magnification. The natural frequencies and vibration patterns of the rod were determined prior to experimentation using an impulse-response modal analysis technique. Positions for the antinodes (maximum displacements) and nodes (zero displacements) were marked on the surface. A small permanent magnet was attached to the free end of the rod and centered beneath an electromagnetic coil driven by a function generator and power amplifier. A 10 mV/g accelerometer was fixed to the bottom surface of the rod (symmetrical placement), and the output amplified and subsequently measured with a spectrum analyzer. An individual major worker ant was extracted from a colony and anesthetized using carbon dioxide gas. While incapacitated a small pin was tethered to the dorsal surfaces of the head and abdomen using glue (only major workers were used because of their favorable physical size). Using a 3-axis micromanipulator the ant was lowered onto the top surface of the rod, ensuring that its legs were coupled naturally to the substrate. A sinusoidal frequency pulse of duration 0.5 seconds was applied to the electromagnetic oscillator in 10 second intervals and resulted in vibrations of the rod. Beginning at sub-threshold amplitudes the voltage was increased in steps of 50 mV until the first leg movements were observed in response to the stimulus. Preliminary trials were conducted using a double-blind test to eliminate any bias in the visual observations. The observations were categorized using the following scheme for statistical purposes: left front leg (LFL) = 1; RFL = 2; LML = 3, etc. Six natural modes of the rod were excited: 42, 250, 650, 1250, 2100, and 3128 Hz. The corresponding substrate accelerations and displacements were calculated from the peak output voltages of the accelerometer and adjusted for amplification. A total of 20 individual major worker ants were stimulated at each frequency studied and the specific leg movements recorded and analyzed. The ambient temperature for all experiments was 23±2 °C. The experimental setup is shown in Fig. 1.
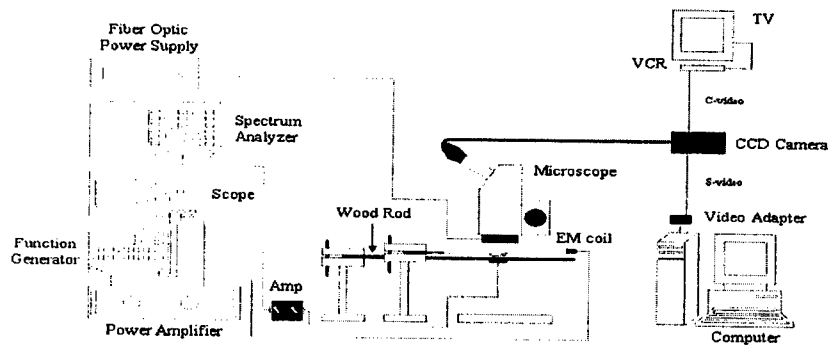
FIGURE 1: Experimental setup for the vibrational stimulation of individual tethered fire ant workers.

PART 1: RESULTS AND DISCUSSION

The vibration sensitivities as a function of the applied frequency are plotted in Fig. 2 (values are reported as mean ± one SEM). The corresponding surface displacements range from 10^{-6} m at 42 Hz to 10^{-9} m at 3128 Hz. The sensitivity thresholds vary over the frequency range studied, with a mean value of 0.78 m/s^2. The data are in agreement with results from previous studies on damp-wood termites (4) and carpenter ants (5). The variations in leg movements for all frequencies studied were determined using one-way ANOVA. From the data, the H value = 540.5 (P < 0.005), and it is concluded that the fire ants responded to the applied stimuli. The similarity in leg movements was determined using repeated measures ANOVA. The F-value = 2.04 was greater than the critical value (P < 0.001), leading to the conclusion that the leg movements were nearly identical in all of the fire ant subjects investigated.
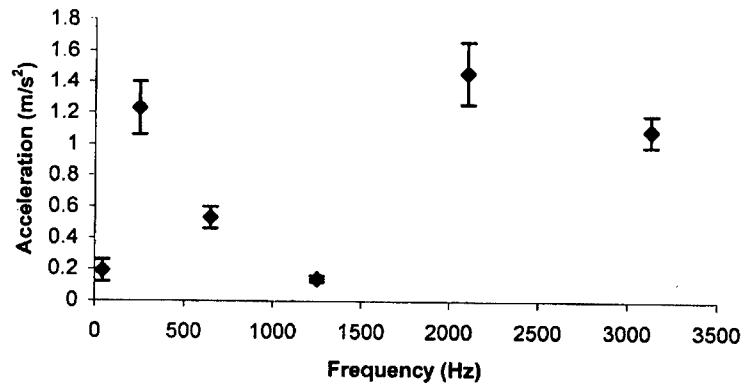
FIGURE 2: Thresholds of vibrational sensitivity for *S. richteri* major workers.

From the data analysis it was concluded that fire ants not only responded to the applied vibrational stimuli but that they also showed similar limb movements. Although the data appear to indicate that the vibratory sensory organs respond to the acceleration component of the substrate vibrations and not the displacement, the debate is still open as to whether the subgenual organs respond to the acceleration or displacement of the substrate. It has been suggested to the authors that a more sensitive means of detecting responses to substrate motions might be achieved by using stimuli of different shapes and/or ramps (i.e. slopes) in order to potentially separate the responses to the different components of the surface motion (6). The variations in the measured accelerations are small over the range of

frequencies studied, but variations in the displacement values are several orders of magnitude. The agreement with data from studies on arthropods demonstrates that this method of visual observation of leg movements can provide a simple, alternative method of measuring the sense of vibrations in insect species. However, the origin of the responses (contractual or reflexive) remains unanswered because the nerve responses were not measured directly using electrophysiological techniques.

PART 2: MATERIALS AND METHODS

This component of the study investigated the behavioral responses of non-tethered fire ant colonies to substrate-borne vibrations. Artificial nesting environments were designed and constructed from polycarbonate boards. These arenas were labeled 1-4 and measured 61 cm × 61 cm × 2.54 cm. Four circular depressions, labeled as dish 1-4, were milled into each board with diameter 1.75 cm. A disc-shaped rare-earth permanent magnet was epoxied to the central bottom surface of each arena. The lower half of the electromagnetic coil was embedded inside a brass cylinder to minimize the strengths of the dynamic magnetic fields during excitation. The arenas were interconnected with tunnels having dimensions 0.95 cm × 0.79 cm to allow for free movement, and the tunnel walls were coated with Fluon® to prevent the ants from escaping. A ruler tape was placed along the top side of each tunnel for measuring the distances moved by the ants in response to the vibrations. Approximately 2.5 grams of fire ants was introduced into dish 1 of arena 1. The fire ants were allowed to acclimate for 10 minutes. To minimize the effects of pheromones emitted from previous colonies of ants, the dish surfaces were rinsed with water and allowed to air dry for 24 hours prior to experimentation (although it was suggested to use both hexane and acetone to remove any residual pheromones, however, these chemicals could potentially dissolve or etch the substrate surfaces, so they were not used). Two modal frequencies very close to those used in Part 1 of the study were selected: 234 Hz and 671 Hz (arena 2, dish 1 and arena 3, dish 1). A sinusoidal frequency pulse of duration 1 second was applied to the electromagnetic oscillator in 10 second intervals and resulted in vibrations of the dish surfaces. A 33-point grid resembling a spider web was marked on the bottom surface of each dish, and surface velocity measurements at each point of the this grid were performed after stimulation of the fire ants using a Polytec PLV model 300 scanning laser vibrometer (1 μm/sec velocity resolution) under similar experimental conditions (Fig. 3).
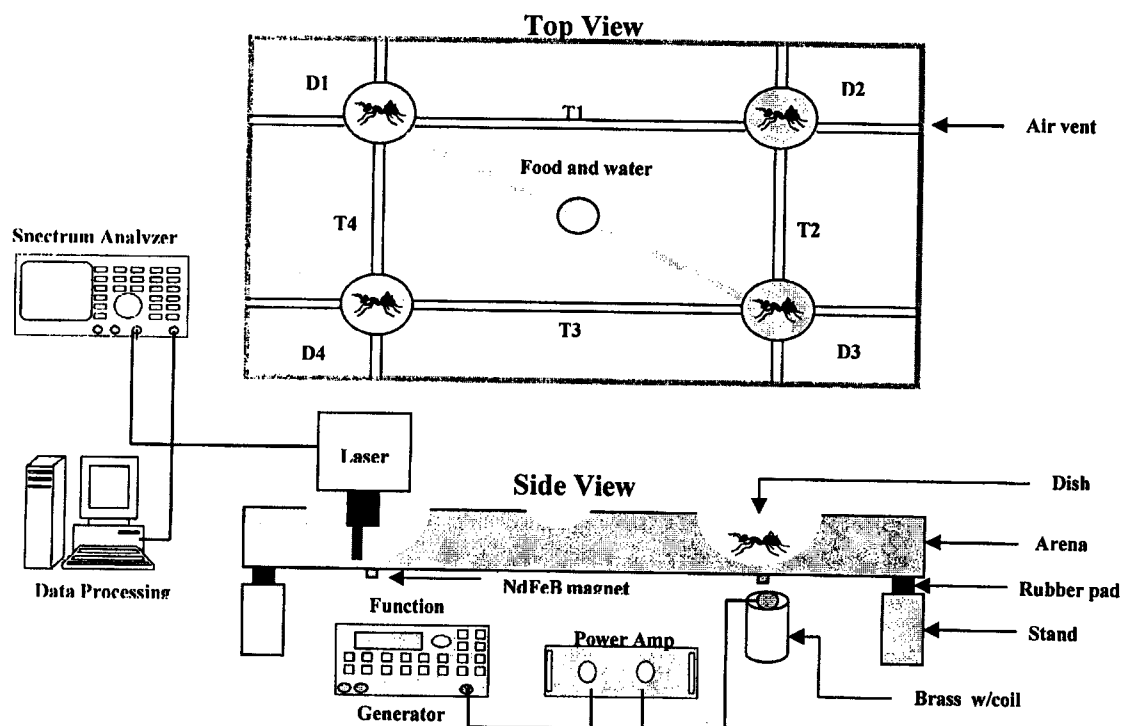

FIGURE 3: Experimental setup for vibrational stimulation of non-tethered fire ant colonies.

PART 2: RESULTS AND DISCUSSION

Surface acceleration measurements were obtained for the original (pre-stimulation) and final (post-stimulation) positions where the fire ants were located (Fig. 4). There was a significant difference in surface acceleration between the original and final locations at 234 Hz (t-value = 6.408, DF=19, P< 0.0001) and at 671 Hz (t-value = 6.044, DF=19, P < 0.0001). Initially the workers were observed to disperse in all directions once the stimuli were introduced. After 1-2 minutes, the minor workers were observed to carry the brood to the relocation site. The major workers actively sought out the source of disturbance, with no specific or preferred directivity observed. The ants relocated to regions of lower substrate displacement/acceleration, except for trials 1, 16, and 20. It was concluded that black imported fire ant colonies are sensitive to substrate-borne vibrations, and that exposure to stimuli of a sufficient threshold stimulates a group behavioral response. The ant colonies were observed to move much more rapidly in response to the frequency mode of 234 Hz (probably due to the higher surface accelerations for this vibration mode at the same input voltage). The vibration levels at the original positions of the clustered ant colonies showed mean acceleration values of 11.3 m/s^2 (234 Hz) and 4.8 m/s^2 (671 Hz), respectively. Vibration levels for the final positions of the ants had mean acceleration values of 2.74 m/s^2 (234 Hz) and 1.25 m/s^2 (671 Hz), respectively. Acceleration values were obtained by averaging the grid points where the ants were distributed. For statistical analysis a paired t-test was used to determine if there was significant difference of the samples before and after treatment. The acceleration data for the final positions are in agreement with the sensitivity thresholds obtained in Part 1 of the study.
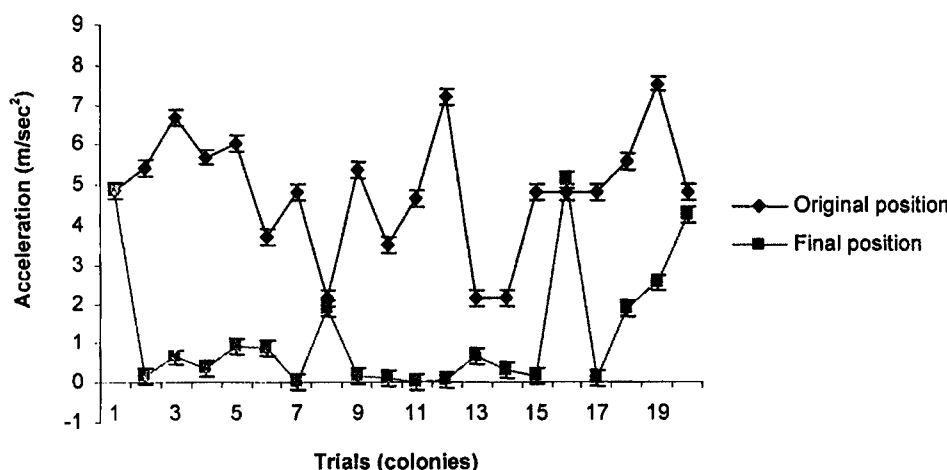
FIGURE 4: Plot of the surface accelerations for both the original and final fire ant positions (f = 671Hz).

PART 3: MATERIALS AND METHODS

The final part of the study involved subjecting fire ant workers to vibrational stimulation while in the presence of attacking phorid flies. This experiment was carried out in the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU) in Mississippi State, Mississippi. A dedicated attack box measuring 244 cm × 56 cm was used (9). Inside of the attack box, three selected tubs measuring 40 cm × 26 cm × 13 cm were each placed in the box among the other tubs. Two of the tubs were considered treatments, and the third was considered a control. One of the treatment tubs employed substrate vibrations, while the other tub used the current inverted-cup system to induce active trailing between the cups. The tubs were randomly distributed among three positions in the attack box in order to eliminate light intensity as a variable that might influence fly distribution (8). A total of 15 unique colonies were used. A group of major workers from one colony was taken, and 1.2 grams of fire ants were placed in each tub. The methodology used here followed the current protocol for rearing flies at the BCMRRU. The fire ant workers were subjected to both substrate vibrations and attacking phorid flies for eight hours duration. The experiment involved cycling through 3 resonant frequency modes of the tubs: 81 Hz, 126 Hz, and 134 Hz. Ants were stimulated for 1 minute duration at intervals of 12 minutes. Observations were made of general ant behavior and phorid attack rates, while fly pupation and emergence were determined microscopically. To determine if there

was any difference in among the treatments, data analysis was done using a one–way ANOVA at a significance level of 5%. Correlations between the number of attacks, parasitization and emergence rates were also determined to find out if there was any relation among the measurements data collected.

PART 3: RESULTS AND DISCUSSION

Clustering of ants was observed in all treatments. However, the vibration tub had similar number of parasitized ants compared to the other treatment tubs (Fig. 5). The control had on overall low parasitization numbers with exception of three trials, which included 1, 7 and 14. In these trials the control showed exceptionally high number of ants parasitized, and this was thought to be due to variables such as location of the control tub in the attack box and the irregular number of flies present during the experiment.
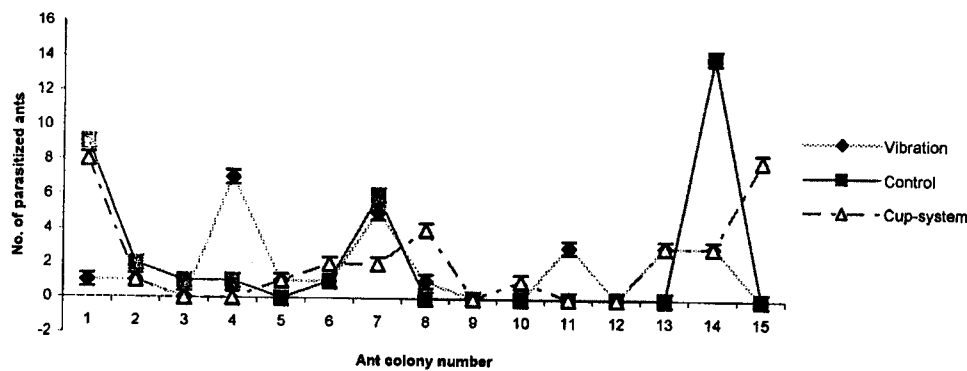
FIGURE 5: The total number of black imported fire ants parasitized in each treatment.

Statistical analysis indicated that there was a significant difference in measurements (number of attacks on ants, emergence of flies and number of ants parasitized) from the tubs, which were labeled as vibration, control and inverted-cup system (F= 6.647, P < 0.00018) (Table 1). There was a strong positive correlation between parasitization and attacks on ants (P < 0.0016) (Table 2). There was also a positive correlation between the number of ants parasitized and the number of flies that emerged (P<0.0021) (Table 2). Overall, there was comparable parasitization in the treatments and the control. The data demonstrated that vibrational disturbances introduced to ants can also lead to increased numbers of attacks by phorid flies by increasing fire ant mobility; ultimately this may result to greater numbers of ants being parasitized.

Table 1: Statistical results for measurements for the number of attacks by phorid flies, parasitization of fire ants and phorid flies emergence.

	DF	F-value	P-value	Power
Treatments	2	0.501	0.6071	0.128
Measurements	2	6.647	0.0018	0.921
Treatments*measurements	4	0.759	0.5540	0.233

Table 2: Correlations of the different measurements that were determined

	Mean diff.	P-value
Parasitized, attack rates	1.886	0.0016
Parasitized, emerged	1.835	0.0021
Attack rates, emerged	-0.051	0.9299

SUMMARY

Longer term application of the vibration-based technique and monitoring of the effects on the host ants should be performed in order to provide additional and conclusive evidence as to which of the specific treatments is most effective in increasing the production of phorid flies. Fire ants are clearly sensitive to substrate vibrations and they sense the surface accelerations and/or displacements in the frequency range of 42 Hz to 3.1 kHz. In response to the substrate vibrations fire ants relocate to regions of low or no disturbance. Exposing ants to substrate vibrations while in the presence of predatory phorid flies is one potential method for improving their mass-production.

ACKNOWLEDGEMENTS

REFERENCES

1. Porter, S.D. and Savignano, D.A. (1990). "Invasion of polygyne fire ants decimates native ants and disrupts arthropod community," Ecology 71, 2095-2106.
2. Roces. F and Tautz, J. (2001). "Ants are deaf," J. Acous. Soc. Am. 109, 3080-3082.
3. Hickling, R. and Brown, R.L. (2000). "Analysis of acoustic communication by ants," J.Acous. Soc. Am. 108, 1920-1929.
4. Kirchner, W.H., Broecker, I., and Tautz J. (1994). "Vibrational alarm communication in the damp-wood termite *Zootermopsis nevadensis*," J. Physiological Entomology 19, 187-190.
5. Fuchs, S. (1976). "The Response to Vibrations of the Substrate and Reactions to the Specific Drumming in Colonies of Carpenter Ants (*Camponotus*, Formicidae, Hymenoptera)," Behav. Ecol. Sociobiol. 1, 155-184.
6. Roces F. (Personal Communication)
7. Porter, S. D. (2000). Host specificity and risk assessment of releasing the decapitating fly *Pseudacteon curvatus* as a classical biocontrol agent for imported fire ants. Biological Control 19, 35-47.
8. Vogt, J.T. (2002). Light intensity affects distribution of attacking Pseudacteon durvatus(Diptera: Phoridae) in a laboratory rearing system. Imported Fire Ant Proceedings, Athens, Georgia, 16-20.
9. Vogt, J.T. and Streett, D. (2003). *Pseudacteon curvatus* (Diptera: Phoridae) Laboratory parasitism, release and establishment in Mississippi.J. Entomol. Sci. 38, 317-320.

Nelson, Roger E., Rice, Stanley A., and Vogt, J.T.

Effects of fire ants on peanut plants

Peanuts are a major crop in the area invaded by fire ants, including Oklahoma, where this study was conducted. Although fire ants can have a positive effect on crop plants (by eating herbivorous insects), their effects on peanuts has been shown to be negative: peanut plants in a field from which ants were excluded grew almost twice as much (aboveground dry weight) as plants exposed to ants. We expect that much of this effect is due to ant damage to peanut seeds soon after planting. In this study, peanut seeds were exposed to fire ants, and the percent damage to each seed calculated. The seeds were then planted in potting soil and grown in a laboratory, and plant weights determined after two weeks. Damage beyond 20% prevented growth prevented growth. Damage between 0 and 20% had a severe effect on growth, especially on root growth. We conclude that even slight damage by fire ants can reduce peanut productivity.

Diurnal patterns of ovipositional activity in two *Pseudacteon* parasitoids (Diptera: Phoridae) in Alabama

V. E. Bertagnolli and L.C. "Fudd" Graham
Department of Entomology and Plant Pathology
301 Funchess Hall ▪ Auburn University, Auburn, Alabama 36849-5413

Introduction

Currently, two species of phorid fly are established in Alabama. *Pseudacteon tricuspis* is established at five sites on populations of the red imported fire ant, *Solenopsis invicta,* and *Pseudacteon curvatus* is established at four sites on populations of a hybrid fire ant (S. *invicta* x *Solenopsis richteri).*

In South America, several species of *Pseudacteon* are often found at the same site and exhibit at least three behaviors that help explain how resources are partitioned (Porter 1997): 1) fly species attack different size fire ant workers (Morrison et al. 1997), 2) they select different periods of diurnal activity (Pesquero et al. 1996) or 3) they attack fire ants engaged in different activities (Orr et al. 1997).

We documented the diurnal activity of the two species of phorid fly that have been introduced into Alabama's imported fire ant populations in an effort to improve current management strategies.

Methods

The first release of P. *tricuspis* was in Macon County in 1999 and the first release of *P. curvatus* was in Talladega County in 2000. The flies have spread over 50 km (ca. 30 mi) and 24 km (ca. 15 mi), respectively, from each release site.

Ants used in the study were collected from the vicinity of the original release sites just prior to field data collection. Ants from four mounds per site were returned to the lab and separated from the soil. Each colony was placed into an individual 52 x 40 x 13 cm tray lined with Fluon®.

The four trays of ants were placed in shady areas of the release sites approximately 8 m (ca. 25 ft) apart. To induce ant pheromone release in order to attract phorid flies, the ants were agitated by shaking the trays. Thirty minutes after agitation, phorid flies were aspirated out of their tray using a double chambered aspirator unit until no flies could be observed in the tray. The flies were transferred from the aspirator to a 14 x 14 x 7 cm plastic holding container via a hole in the container lid. Carbon dioxide was introduced into the holding container to induce fly knock down. Upon knock down, the lid was removed from the container and flies were counted. After fly count, the container was placed in the shade to allow for fly recovery and release. The tray was shaken again and the collection process was moved to the next box. The collection process started approximately two hours following sunrise (ca. 8:30 am) and was repeated every 30 minutes until flies ceased coming to the trays approximately 12-13 hours following sunrise (ca. dusk).

Results

Mean daily activity patterns for *P. tricuspis* in Alabama were similar to those found by Pesquero et al. (1996) in Brazil, with mean peak activity occurring during mid-

day (fig. 3). In Brazil, activity was greatly reduced 12 hours following sunrise. However, in Alabama, *P. tricuspis* were still active at this time (ca. 6 p.m.), extending ovipositional activity into hours when *Pseudacteon litoralis* is usually active in Brazil (Pesquero et al. 1996).

Peak activity for *P. curvatus* occurred later in the afternoon 10–11 hours following sunrise with moderate activity observed from four to twelve hours past sunrise (fig. 4). This pattern was similar to the pattern *P. litoralis* exhibited in Brazil (Pesquero et al. 1996) where peak activity occurred 10-12 hours following sunrise with slightly lower fly activity two to four hours following sunrise.

References

Morrison, S. W., S. D. Porter and L. E. Gilbert. 1999. Sex ratio variation as a function of host size in *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). Biol. J. Linn. Soc. 66:257-267.

Orr, M. W., S. H. Seike and L. E. Gilbert. 1997. Foraging ecology and patterns of diversification in dipteran parasitoids of fire ants in south Brazil. Ecol. Entomol. 22: 305-314.

Pesquero, M. A., S. Campiolo, H. G. Fowler and S. D. Porter. 1996. Diurnal patterns of ovipositional activity in two *Pseudacteon* fly parasitoids (Diptera: Phoridae) of *Solenopsis* fire ants (Hymenoptera: Formicidae).

Porter, S. D. 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol.
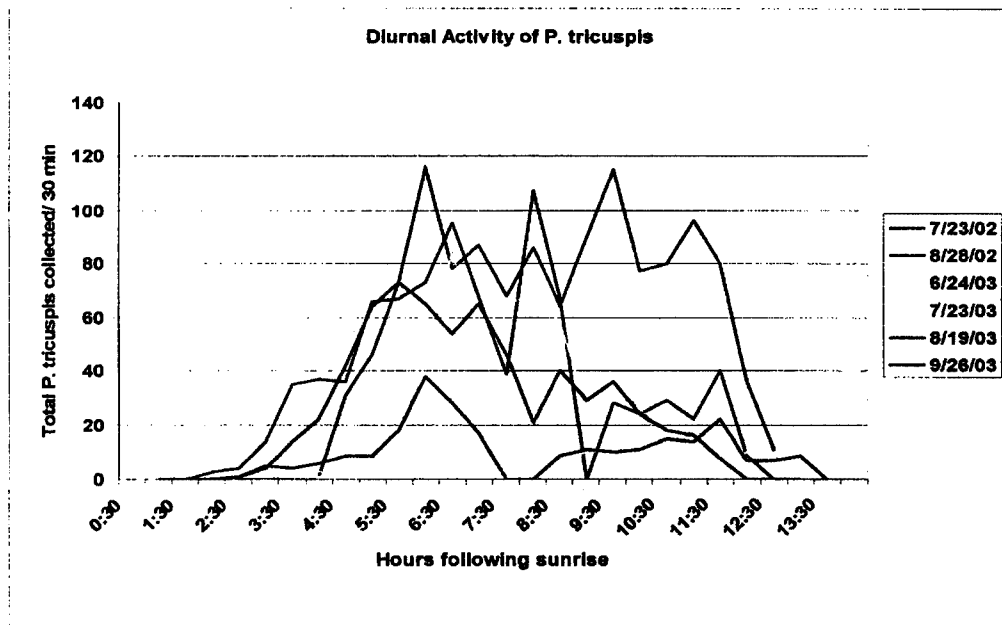
Figure 1. Diurnal activity patterns of *P. tricuspis* on six collection dates in Macon County, Alabama.
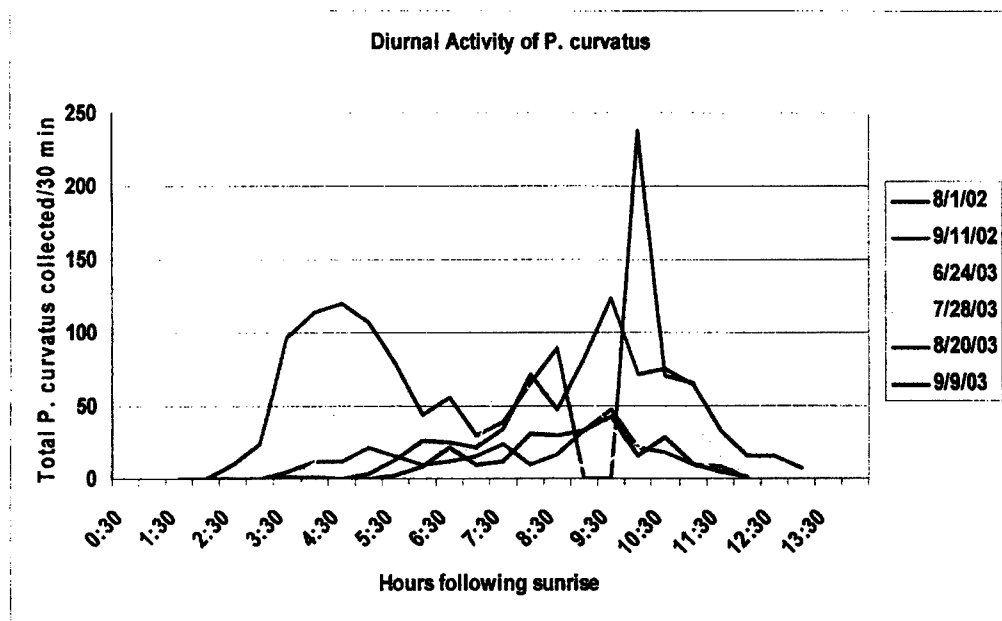
Figure 2. Diurnal activity patterns of *P. curvatus* on six collection dates in Talladega County, Alabama.
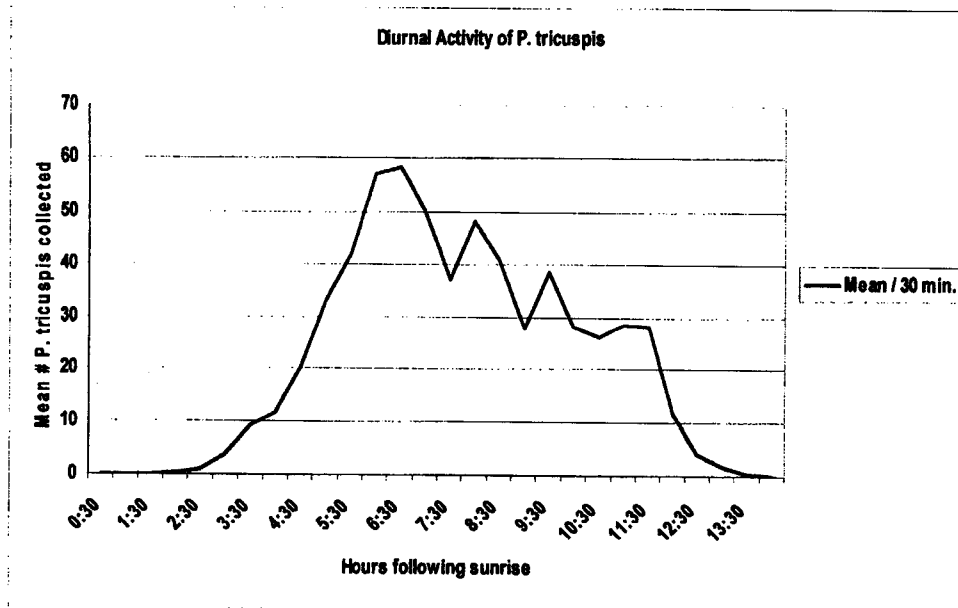
Figure 3. Mean daily activity patterns of *P. tricuspis* in Macon County, Alabama.
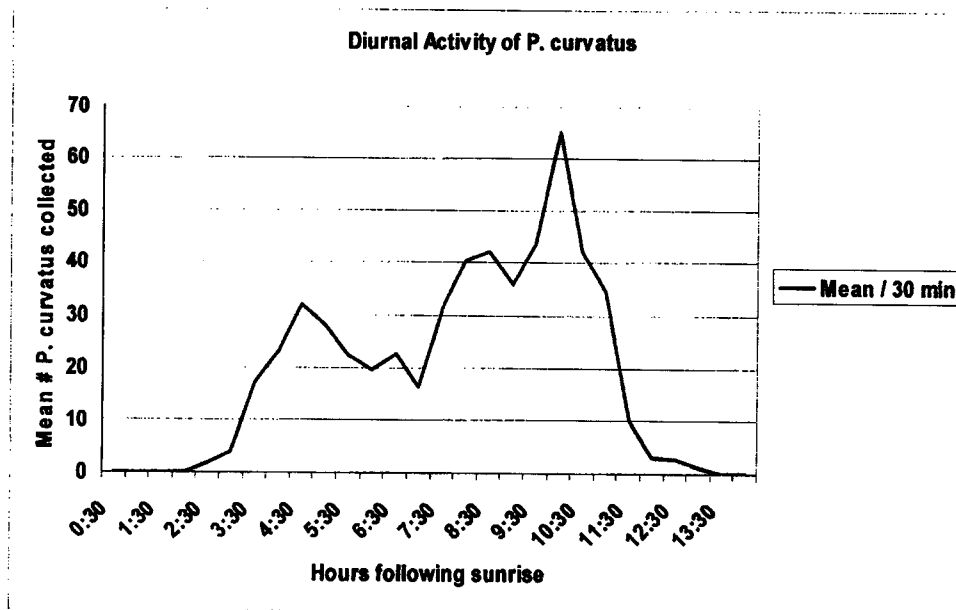
Figure 4. Mean daily activity patterns of *P. curvatus* in Talladega County, Alabama.

INTERACTIONS BETWEEN THE PYRAMID ANT (*Dorymyrmex flavus* Mc Cook) AND THE RED IMPORTED FIRE ANT

Alejandro Calixto, Charles Barr and Marvin Harris
Department of Entomology, Texas A&M University
acalixto@tamu.edu

The introduction of the red imported fire ant *Solenopsis invicta* Buren has resulted in the reduction of many native ant fauna in the US through direct and indirect competition (Camilo and Phillips 1990; Porter and Savignano 1990; Jusino-Atresino and Phillips 1994; Wojcik 1994; Wojcik et al 2001). *Dorymyrmex flavus* Mc Cook is one that despite of increased numbers of fire ants seems to be adapted in some instances and to survive the invasion leading to an "apparent" coexistence between these two species, and in many cases they have been observed by the authors nesting in close proximity.

There have been several observations recounting interactions between these two species in the field. *D. flavus* has been recorded attacking newly mated queens and males (Whitcomb et at 1973, Nickerson et al 1975), and also observed on refuse piles where *Dorymyrmex* accumulates a high proportion of fire ant remains (Hung 1974). These anecdotal observations indicated some kind of interactions occurring between these species.

Other interactions between native ant fauna and fire ants concerns the use of baits for the control of fire ants in managed and unmanaged areas, the effect on local native ant fauna, how they respond to the reduction /suppression of fire ant, and what impact this may have on these assemblages. I studied the effect of Extinguish™ bait treatment on populations of D. *flavus* and *S. invicta* and the advantages and disadvantages on management of fire ants and conservation of native ant fauna, in this case *Dorymyrmex.*

The objectives of this study were first, to determine the effect of Extinguish™ bait treatment on the invasive ant *S. invicta* and native *D. flavus,* second, to explore the mechanisms involving coexistence of these species pre-, during and post-bait treatment, and third, to assess possible antagonistic behaviors of the pyramid ant towards *S. invicta* that may lead in buffering reinvasion after treating with baits.

The study was conducted in a commercial pecan orchard in Robertson Co., Texas where we established two treatments replicated four times by randomly assigning each replication to a 3.3 acres block. Treatments were 1), untreated control and 2), Extinguish™ bait treatment applied twice in year 2000 (April, October), once in year 2001 (June) and no treatment at all in year 2002. Several methods were used to monitor *Dorymyrmex* and *Solenopsis* during these two years in each treatment. Mound counts using a transect of 1/8 of an acre were used on each one of the treatments to determine the density of these two species. Mound counts were done four times in 2000 (a pre-count in march, post-counts in June, July and December), twice in 2001 (April, October) and once in July 2002. Pitfall traps monitored on a weekly basis were used to determine relative abundance of the species involved; traps were placed in April 2000 and run for two years with the last sample being on June 2002 (four traps per treatment). Weekly samples were collected and processed. Baited vials containing candy and cat food were placed on a weekly basis to determine foraging behavior, vials were exposed for 24

hours, then collected and processed. 64 vials were used per treatment and as with the traps, they were run for 2 years. Additional observations on *Dorymyrmex* nests were done, 10 middens of D. flavus were collected randomly, brought back to the lab and processed, and all samples were inspected for remains of S. invicta, classified and recorded. A week later midden samples were collected from the same nests again. Video recording on the behavior of *D. flavus* in 3 mounds located at a distance of 1 meter away from fire ant nests; recording sessions were done for intervals of ten minutes every twenty minutes for 4 hours in the afternoon, 6 days in September and one in October 2003. Later on, videos were inspected and the frequency of *Dorymyrmex* involving a behavior with fire ants was recorded. Ants bring fire ants in the nest and remove fire ants from the nest during these intervals, and notes on antagonistic behavior towards fire ants were also taken.

Results of mound counts indicated a consistent reduction of *Solenopsis invicta* mounds following Extinguish[TM] treatment and a consistent increase in *Dorymyrmex* nests. A Mann-Whitney t-test was performed by sample day comparing treated vs. untreated (significantly different ($P>0.0.5$ $\alpha=0.05$) for *S. invicta* and *Dorymyrmex* on samples followed first treatment in year 2000 and the first count in 2001. Clearly Extinguish[TM] is having a positive effect on the reestablishment of *Dorymyrmex* nests when fire ants are suppressed. Pitfall traps and baited vials showed the same trend as the mound counts. A reduction of the abundance of foragers in fire ant nests following bait treatment and an increase in abundance and foraging behavior of *D. flavus* workers in blocks treated for fire ants occurred. A GLM Univariate test was performed to test the response of *Solenopsis* and *Dorymyrmex* to treatments and sample week. Data were separated for years being significantly different ($P>0.0.5$ $\alpha=0.05$) for both species in pitfall traps, and for baited vials, significant differences ($P>0.0.5$ $\alpha=0.05$) were just found on fire ants foraging on these vials. Observations on middens showed that +90% of ant remains found were fire ants, with heads, thoraxes, abdomens and the complete bodies observed on the middens. Fire ant accumulation appeared to be rapid judging by numbers collected during the 7 day sampling interval. These data were also consistent with the video, where *Dorymyrmex* were observed interacting with fire ants and depositing remains in the middens. Direct observation of *Dorymyrmex* aggression towards fire ants was not observed, although data were strictly collected on *D. flavus* mounds, and antagonistic behavior may have occurred outside of this perimeter. Whether *Dorymyrmex* are scavenging dead fire ants or killing them is unknown, but it is very likely both events are happening. The numbers of dead fire ants collected on middens represent a significant amount of protein in this particular environment

In conclusion, we found that Extinguish[TM] resulted in increased densities of *Dorymyrmex*, with no adverse effects observed on this native ant. *Dorymyrmex* appears capable of resisting fire ant re-invasion of treated areas and may continue occupation and survival there for many months, or even years, based upon our observations.

References

Camilo, G. R. and S. A. Phillips. 1990. Evolution of ant communities in response to invasion by the fire ant *Solenopsis invicta*. In Applied Myrmecology, a world perspective. R. K. Vander Meer, K. Jaffe, and A Cedeno (Eds). Westview Press, Boulder, CO. Pp. 190-198.

Hung, A. C. F. 1974, Ants recovered from refuse pile of the Pyramid ant *Conomyrma insana* (Buckley) (Hymenoptera: Formicidae). Ann. Entomol. Soc. Amer. 67(3):522-523.

Jusino-Atresino, R. and S. A. Phillips, Jr. 1994. Impact of Red Imported Fire Ants on the ant fauna of central Texas. In: Exotic Ants: Biology, Impact, and Control of Introduced Species. Ed. David F. Williams. Colorado-Boulder. Westview Studies of Insect Biology. Pp. 260-268.

Nickerson, J. C., W. H. Whitcomb, A. B. Bhatkar and M. A. Naves. 1975. Predation of founding queens of *Solenopsis invicta* by workers of *Conomyrma insana*. Florida Entomol. 58:75-72.

Porter, S. D. and D. A. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology, 71(6): 2095-2106.

Whitcomb, W. H., A. Bhatkar and J. C. Nickelson. 1973. Predators of Solenopsis invicta queens prior to successful colony establishment. Environmental Entomology 2(6): 1101-1103.

Wojcik, D. P. 1994. Impact of the Red Imported Fire Ant on native ant species in Florida. In: Exotic Ants: Biology, Impact, and Control of Introduced Species. Ed. David F. Williams. Colorado-Boulder. Westview Studies of Insect Biology. Pp. 269-281.

Wojcik, D. P., C. R. Allen, R. J. Brenner, E. A. Forys, D. P. Jouvenaz and R. S. Lutz. 2001. Red imported fire ants: impact on biodiversity. American Entomologist 47(1): 16

Assessment of Landscape-level Impacts of Red Imported Fire Ants on Native
Invertebrate Communities in Pine-dominated Forests

Keri E. Landry[1], Linda M. Hooper-Bùi[2], Michael J. chamberlain[1], and Lee A. Womack[1]
[1]Louisiana State University, School of Renewable Natural Resources, 227 SRNR
Building, Baton Rouge, La 70803; 225-578-7718; klandr5@lsu.edu.
[2]Louisiana State University, Department of Entomology, 404 Life Science Building,
Baton Rouge, La 70803

Since the accidental introduction of red imported fire ants *(Solenopsis invicta* Buren,
RIFA) into Mobile, Alabama in the 1930's, the invasion of this species into other areas
across the southeast has increased drastically. Most research on effects of RIFA on
vertebrates and invertebrates have focused on small scale areas and single species. We
suspect that populations of RIFA have an effect on native arthropod communities,
therefore decrease biodiversity. We examined established populations of RIFA in
relation to native invertebrate communities in longleaf pine and pine-hardwood forests.
We evaluated the efficacy of using Amdro® to control RIFA and determined the effect of
RIFA predation on arthropod communities. RIFA suppression began in April 2003 and
occurred every six months. Arthropod abundance and diversity were measured within
each forest type for 2 consecutive years, 1- year pre-treatment and 1-year post-treatment.
We hypothesize that a significant reduction in abundance and distribution of RIFA in
stands treated with Amdro® corresponds with greater abundance and diversity of
arthropods observed within each forest type. We were effective in suppression of RIFA
on treated stands versus controls. In the pine-hardwood forest site, densities of Acari
were significantly higher between stands treated with Amdro® versus untreated controls.
Densities of Araneae were significantly higher between treatments in the longleaf pine
forest site. Our results indicate that particular invertebrate orders may be negatively
affected by RIFA through predation and competition.

Effect of fire ant presence in mammal traps on bait theft and trap success in two ecosystems in Louisiana

L.M. Hooper-Bùi[1], M.J. chamberlain[2], and J. Constible[2]

[1]Louisiana State University, Department of Entomology, 404 Life Science Building, Baton Rouge, La 70803 225-578-1832;

[2]Louisiana State University, School of Renewable Natural Resources, 227 SRNR Building, Baton Rouge, La 70803: 225-578-4241.

For more than 60 years there has been a growing awareness and interest in wildlife conservation, especially preventing loss of wild birds and other animals to insect predators and parasites (Stoddard 1932; Atwood et al. 1978). Of particular concern are reported losses of vertebrates to ants, specifically *Solenopsis invicta* Buren, red imported fire ants. Some species of ants are opportunistic predators or scavengers, attacking several species of vertebrates, especially ground nesting birds (Stoddard 1931; Travis 1938a,h; Emlen & Glading 1945; Johnson 1961; Kroll et al. 1973; Parker 1977; Jackson & Jackson 1985a,b; Ridlehuber 1982; Masser & Grant 1986, Pederson et al. 1996).

Many studies have named *S. invicta* as the dominant invertebrate predator in areas they inhabit and that they subsequently reduce invertebrate diversity and impact ground dwelling or nesting species (Harris and Burns 1972; Burns and Melancon 1977; Vinson and Sorensen 1986). Vinson and Sorenson (1986) report that *S. invicta* interfere with hunting and reduce the public's utilization of infested parks and recreation areas. Additionally, *S. invicta* may reduce populations and the success of endangered species such as least terns and other ground nesting birds such as northern bobwhite quail (Littlefield 1987; Lockley 1995).

Few studies have been published on impacts of *S. invicta* predation on ground nesting mammals in their natural settings. Some manipulative studies have shown no apparent effect on vertebrate populations by *S. invicta*, whereas others have noted detrimental effects (Lechner and Ribble 1996). In an enclosed pen closely resembling a natural setting, cottontail litters existed near *S. invicta* mounds with > 25 % attacked by *S. invicta* (Hill 1969). An earlier study with cotton rat litters also was conducted in small pens surrounded by *S. invicta* mounds; however, no observations of RIFA predation occurred (Johnson 1961). Smith ct al. (1990) stated that certain small mammal species would avoid places where RIFA are abundant, thus added a negative bias to trap success. This was examined by Mitchell et al. (1996) who found that trap catch was unchanged on insecticide treated sites. However, they did find that mutilation rates in the traps were lower in the treated areas.

In Louisiana, we observed fire ants stealing mammal bait and attacking the mammals in the traps. It is possible that a contact insecticide treatment zone around the mammal traps might protect the traps from ant invasion. Treatment zones or insecticide barriers are commonly used by homeowners and pest management professionals to prevent ants from entering the structures (Pranschke et al. 2003). Bifenthrin has been shown to be non-repellent to fire ants but it will kill ants that contact it within 15 minutes and severely impair them sooner (Richman and Hooper-Bùi 2003). Pranschke et al. (2003) demonstrated that bifenthrin sufficiently protected a food attractant placed in the center of a circle with a treated radius. A radius of 0.3 m only provided a week of protection.

One of the objectives of this study was to examine how fire ants presence in the trap affect mammal trap success in early and late successional pine plantation ecosystems. We investigated these ecosystems because fire ants differ in abundance and activity in ecosystems at different successional stages. We investigated whether creating a barrier treatment zone around mammal traps would (1) reduce bait theft by fire ants, (2) increase trap success, and (3) provide protection for four trap nights. The number of mammals trapped in treated traps was compared with those trapped in untreated traps.

We conducted research on two areas in central Louisiana. The first study area was the 700 ha Idlewild Research Station owned by the Louisiana State University Agricultural Center located south of Clinton, Louisiana. The second study area was the 17,700 ha Sherburne Wildlife Management Area located east of Krotz Springs. Sherburne lies within a bottomland hardwood forest in the Atchafalaya Basin.

We live-trapped small mammals to quantify effectiveness of bifenthrin in protecting traps from ants and to estimate relative abundance and species diversity. We used Sherman live traps baited with peanut butter and oatmeal on a 5 x 5 grid system with each grid containing 25 traps. Traps were spaced approximately 10 m apart. We placed a 1 m barrier of the granular contact insecticide bifenthrin (Talstar PL®, 0.6% bifenthrin FMC Corporation; 1.97g formulated material /3.14m^2, 2.087 kg of formulated material / 92.88 m^2, 4.6 lb formulated product/ 1000 ft^2, or 4.2 g active ingredient/ 92.88 m^2) around every other trap after it was initially set. We sampled six grids on Idlewild and three on Sherburne, and each grid was sampled for four consecutive nights. Each grid was separated far enough from the others to ensure that mammals could not be trapped at more than one grid. All traps were checked each morning. Captured mammals were weighed, sexed, aged, and marked using toe clipping (with analgesic and anesthetic before and after clipping to minimize pain and infection (AD HOC 1985, AD HOC 1987)). Trapping and marking procedures were approved by Louisiana State University Institutional Animal Care and Use Protocol Number A-00-02.

We investigated whether the bifenthrin treatment zones protect the trap from mammal bait theft by fire ants. Unprotected mammal traps had bait stolen by fire ants twice as often as those protected by a bifenthrin treatment zone (Mean±SEM, 0.84±0.024 vs 0.44±0.078; N=18, t=8.376, P<0.001). However, we also examined the effectiveness of bifenthrin over the four trap nights and found that bifenthrin decreases in efficacy. On the first day, 91±3.4% (Mean± SEM) of the treated traps were protected from fire ants, but by the fourth day only 77±3.2% (Mean± SEM) of traps were protected from fire ants. Univariate and multivariate repeated measures analysis of the transformed means revealed significant differences with increased protection from fire ants provided on the first day (F=5.67, df=3, P=0.004) and a polynomial test indicated that the relationship over time was roughly linear (F=15.89, df=1, P=0.004). Large swarms of fire ants were more likely to steal bait than swarms with few fire ants (F=28.547; df=2, P<0.001). When we examined the number of ants associated with breaches of the bifenthrin treatment zone, we discovered that there were only a few ants in the traps (F=3.37, df=3, P=0.024) and that there were no large swarms of ants in treated traps (P>0.05). When non-target ants were examined, there was no effect of treatment or day on whether any non-fire ants were found in the traps, although there were often large amounts of non-target ants in the traps. It is not known whether those ants affect mammal trap catch.

Fire ant activity on the mammal grids was highly correlated with bait stolen from the treated traps. When we used a Pearson's correlation to associate the proportion of treated traps with bait and the proportion of traps with fire ants present, the traps with high numbers of ants were more likely to have the bait stolen (Bartlett Chi-square=9.94, df=1, P=0.002). However, the presence of fire ants in treated traps was not correlated with levels of fire ant activity on other portions of the mammal grids (Bartlett Chi-square=0.812, df=1, P=0.37). Fire ant activity on the mammal grids was weakly correlated with the presence of fire ants in untreated traps (Bartlett Chi-square=2.694, df=1, P=0.101). We captured 38 small mammals during our study and although capture success was considered low, nearly twice as many mammals (n = 24) were captured in treated traps than nontreated (n = 14).

Bio-Control

Infection of Red Imported Fire Ant Colonies with the Microsporidiurn *Vairimorpha invictae*

David H. Oi, Juan A. Briano[1], and David F. Williams
USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology
1600 SW 23rd Drive, Gainesville, Florida 32608

[1]South American Biological Control Laboratory, Bolivar 1559 (1686)
Hurlingham, Buenos Aires province, Argentina.

Summary

Vairimorpha invictae is a microsporidium pathogen that infects red imported fire ants, *Solenopsis invicta* Buren, and was first described by Jouvenaz and Ellis (1986). Surveys of fire ants in South America indicated that this pathogen had a moderately low prevalence being found in 2.3% of 2,528 colonies surveyed (Briano and Williams 2002). In a survey of black imported fire ants, *Solenopsis richteri* Forel, and *Solenopsis quinquecuspis* Forel in Buenos Aires province, Argentina, Briano et al. (1995) reported *V. invictae* in *1%* of the colonies compared to **8%** (N=1,836) for another microsporidium pathogen of fire ants *Thelohania solenopsae* Knell, Allen, & Hazard. Preliminary results from field infections of *V. invictae* in *S. invicta* suggested that this pathogen could reduce colony populations and had the potential to be an effective biological control agent (Briano et al. 2002). Attempts by Briano (unpublished data) and Jouvenaz and Ellis (1986) to infect fire ant colonies in the laboratory were unsuccessful. To further assess the potential of *V. invictae* as a biological control agent for introduction into the U.S., our objective was to infect *S. invicta* colonies under laboratory conditions.

Based on surveys conducted by Briano, *V. invictae* infected *S. invicta* colonies were collected near San Javier, Santa Fe province, Argentina in 2003 and transported to quarantine facilities at the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida. Incipient colonies reared from newly-mated, *S. invicta* queens that contained approximately 400 pieces of brood, 30 workers, and 1 queen, were inoculated with brood from *V. invictae* infected colonies. Inocula consisted of either 10 live, 4 instar larvae or 10 live, non-melanized pupae from colonies where infection rates of brood were 100%. From the larval inoculations, 2 of the 5 colonies became infected. Introductions of the non-melanized, pupae also resulted in infections of 2 of 5 colonies. In addition, 2 of 6 colonies became infected after introducing dead adult worker caste ants collected from *V. invictae* infected colonies. Infected colonies from the larval and pupal inoculations had less brood (81%) and adults (92%) than control colonies 28 weeks after inoculation. For the infected colonies inoculated with dead adults, queens were dead 14-16 weeks after inoculation and subsequent colony decline followed. In contrast, queens from non-inoculated colonies were still alive after 33 wk.

Infections were also obtained from 10 of 12 inoculations using either: larvae, white pupae, or melanized pupae derived from colonies that were previously infected in the laboratory. Infections were again obtained in incipient colonies from 6 of *6* larval, 1 of 3 non-melanized pupal, and 3 of 3 melanized pupal inoculations. Inoculations of larger *S. invicta* colonies containing 10,000 – 15,000 workers, 15 ml of brood, and 1 queen, using a mixture of larvae and pupae from *V. invictae* infected colonies resulted in 1 of 15 colonies being infected. The queen in the infected colony died by the 28' week after inoculation and brood and worker populations declined by 100 and 70%, respectively.

Acknowledgments

References Cited

Briano, J. A. and D. F. Williams. 2002. Natural occurrence and laboratory studies of the fire ant pathogen *Vairimorpha invictae* (Microsporida: Burenellidae) in Argentina. Environ. Entomol. 31: 887-894.

Briano, J. A., D. P. Jouvenaz, D. P. Wojcik, H. A. Cordo, and R. S. Patterson. 1995. Protozoan and fungal diseases in *Solenopsis richteri* and *S. quinquecuspis* (Hymenoptera: Formicidae), in Buenos Aires province, Argentina. Fla. Entomol. 78: 531–537.

Briano, J. A., D. F. Williams, and D. H. Oi. 2002. Long term effect of the fire ant pathogens *Vairimorpha invicta* and *Thelohania solenopsae* in Argentina. Proc. 2002 Imported Fire Ant Conf., Athens, Georgia, March 24-26, 2002. p. 15.

Jouvenaz, D. P. and E. A. Ellis. 1986. *Vairimorpha invictae* n. sp. (Microspora: Microsporida), a parasite of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). J. Protozool. 33: 457-461.

Occurrence and distribution of *Thelohania solenopsae* in Louisiana red imported fire ant *(Solenopsis invicta)* populations.

Maynard L. Milks, James R. Fuxa, and Arthur R. Richter

In April 2003, we conducted a statewide survey of S. *invicta* populations to determine the pathogens infecting this insect in Louisiana. The ultimate goals were to establish baseline data for S. *invicta* pathogens, analyze associated environmental data for factors that might affect prevalence, and identify pathogens with potential for being developed as biological control agents.

In all, we surveyed 165 sites (1309 colonies) ranging from lawns in residential neighborhoods, to highway right-of-ways, to agricultural fields. At each site, we established 0.05 ha plot, determined the density and size of S. *invicta* colonies, and collected ant samples from a maximum of 10 colonies. We also collected soil and vegetation samples at each site. In the laboratory, the ant samples were first observed for gross symptoms of disease. This was followed by microscropic examinations of wet mounts and trichrome stains of ant smears. Finally, the samples were screened with the polymerase chain reaction with disease-specific primers.

Here, we present preliminary results of our survey with emphasis on the occurrence and distribution of *Thelohania solenopsae*, a microsporidian pathogen of S. *invicta,* in relation to ant social form.

To date, the microsporidium was detected in 15 of 85 sites in Louisiana. Eight of the *Thelohania*-positive sites were loosely concentrated in east central Louisiana; the remaining seven were isolated and scattered elsewhere in the state. Four of the positive sites had 90-100% infection among colonies. All four infection x social form possibilities were detected: uninfected polygyne, infected polygyne, uninfected monogyne, and infected monogyne colonies.

Variability in the occurrence of *Thelohania solenopsae* in *Solenopsae invicta* population in Oklahoma

Vedham Karpakakunjaram and Russell E. Wright

Department of Entomology and Plant Pathology
Oklahoma State University
Stillwater, OK 74078

Corresponding Author:

Vedham Karpakakunjaram

Department of Entomology and Plant Pathology

Division of Agricultural Sciences and Natural Resources

127 Noble Research Center

Oklahoma State University

Stillwater, OK 74078

Ph: (405) 744-7502

Fax: (405) 744-6039

Email: karpaka@okstate.edu

Abstract

The absence of natural enemies is one of the key factors that enabled Red Imported Fire Ant, *Solenopsis invicta* to flourish and spread in the southern parts of the United States. *Thelohania solenopsae*, a microsporidian parasite and *Pseudacteon tricuspis*, a phorid fly are two major natural enemies of fire ants in South America. Recent investigations have focused on *T. solenopsae* as a potential biocontrol agent for S. *invicta*. Here, we report the patterns of occurrences of *T. solenopsae* in the populations of *S. invicta*, in Oklahoma. For the first time in Oklahoma, fire ants collected in October 2002 tested positive for the microsporidian pathogen as confirmed by Polymerase Chain Reaction and modified Trichrome staining methods at the two sites in Bryan Co., OK. About 85% to 90% of the *S. invicta* samples collected in October 2002 from Bryan Co. sites were infected with *T. solenopsae*. However, it declined to about 30%-33% in May 2003 and again increased to 47%-65% in September 2003. All fire ant samples from McCurtain County were devoid of *T. solenopsae* spores in 2002 and 2003. The fluctuation in the occurrence of the parasite in the fire ant populations probably indicates that the parasite is yet to establish significantly due to unfavorable weather conditions in Oklahoma. In addition, the turnover of infected and healthy fire ant colonies after every winter in Oklahoma may be high which may keep the infection rate low. Our future investigation will focus on the patterns of *T. solenopsae* spread among the fire ant population in Oklahoma and the response of the fire ants towards the establishment of the parasite.

Introduction

Since the accidental introduction in the 1930s, *Solenopsis invicta* has emerged as one of the major invasive insect species in the United States. It infests over 112 million hectares in the United States (Lofgren, 1986) and impacts the environment in numerous ways. *S. invicta* is more abundant in the United States than in its natural habitats in South America (Porter *et al.*, 1992), probably due to lack of natural enemies in the introduced habitats. Several workers have recently begun to focus on using sustainable control methods, which mainly includes biological control agents. *Thelohania solenopsae*, a microsporidian obligate intracellular pathogen has been an effective natural enemy of the fire ants in Argentina (Briano *et al.*, 1995). *T. solenopsae* declines the egg production, queen weight and survivability of queens and workers of *S. invicta* (Oi and Williams, 2002). In addition, Cook (2002) observed that the colonies infected with *T. solenopsae* had lesser mound volume than the uninfected colonies. Also, the colony densities were positively correlated to percentage of infection.

In 1998 and 2000, spores of *T. solenopsae* were released in Bryan Co., OK, and Carter Co., OK, respectively. However, the status of the pathogen remained uncertain. Since October 2002, we have been monitoring the distribution patterns of *T. solenopsae* at three sites in Oklahoma. Two of these sites are in Bryan Co., OK and the third is in McCurtain Co., OK and are part of our Area-wide fire ant suppression project. The source of *T. solenopsae* spores in Oklahoma is not known however, it will be interesting to track the movements of Red Imported Fire Ant (RIFA) along with the spread of *T. solenopsae*, in Oklahoma.

Materials and Methods

Fire Ant samples were collected from three sites – two from Bryan Co., OK and the other from McCurtain Co., OK. These sites are ranches where we conduct our Area wide Suppression project studies. Each site has several $1/8^{th}$-acre plots (Table 1) and samples were collected from a maximum of 4 mounds in each plot that had fire ant mounds. The samples were tested for the presence of *Thelohania solenopsae* spores using PCR and modified Trichrome staining.

Polymerase Chain Reaction:

Fifteen to thirty ants were ground with 500µl TBS using a disposable pestle. To this homogenate, 0.1mm glass beads were added up to three-quarters of the tube and beaten at maximum speed for 15 seconds, in a bead beater. The tubes were immediately transferred and kept in 95°C water bath for 5 min. The samples were spun for 10sec at 18000g and the supernatant containing genomic DNA of *T. solenopsae* was collected. 2µl of the total DNA was added to the mixture of *T. solenopsae* specific primers - Msp1a and Msp4b (1µl each) and Ready-to-go- PCR beads. This mixture was made up to 25µl with sterile distilled water and the PCR reactions were set following the protocol of Snowden *et al.*, 2002. PCR products were separated on 2% agarose gels and visualized by Ethidium bromide staining. For all experiments, positive (*T. solenopsae* DNA) and negative (PCR reaction mixture without total DNA) controls were also run along with treatments.

Trichrome staining:

Fifteen to thirty ants were ground with 500µl TBS using a disposable pestle. About 15µl of the ground solution is placed on labeled glass slide and air-dried. The slides are stained using the modified Trichrome staining protocol of Kokoskin *et al.* (1994). The slides were observed under a light microscope at 400x and 1000x magnifications for the spores of the pathogen (Fig 1a&b).

Results and Discussion

Two of the three sites had *T. solenopsae* infections in the RIFA samples. The source of *T. solenopsae* in 2002 and thereafter in Oklahoma is unknown, as two earlier attempts (1998 and 2000) to establish this pathogen in *S. invicta* mounds failed. All the RIFA samples collected from the site in McCurtain County were uninfected. In Bryan County, this microsporidian pathogen was present in 85.7% and 89.5% (Table 1) of the samples analyzed at Adam's and Bowles' Ranches, respectively, in 2002. There was a distinct reduction in the percent of infection at these two sites in spring 2003 (30% and 33.3% respectively). Interestingly, the percent of infection increased again by fall 2003 to 70.6% at Adam's Ranch and 58.8% at Bowles' Ranch (Table 2). This pattern of infection creates an interesting scenario wherein the cause for the fluctuation could potentially be investigated by a long term monitoring of these sites.

We hypothesize that the reduction in the microsporidian infections in spring could be due to the winter conditions in Oklahoma. This cold weather may be severe for the typically tropical *T. solenopsae*. In such a case, every spring, there is turnover of infected queens from neighboring warmer regions. The status of *T. solenopsae* in the RIFA

population of Oklahoma is an ideal model to study the interactions between the two species and the strategies they may adopt to establish.

Acknowledgments

References

1. Briano, J., Patterson, R., and Cordo, H. 1995. *Environ. Entomol.* **24**, 1328-1332.

2. Cook, T. J. 2002. *Environ. Entomol.* **31**, 1091-1096.

3. Jouvenaz, D.P., Lofgren, C.S., and Banks, W.A. 1981. *Ann. Entomol. Soc. Am.* **27**, 204-208.

4. Kokoskin, E., Gyorkos, T.W., Camus, A., Cedilotte, L., Purtill, T., and Ward, B. 1994. *J. Clin. Microbiol.* **32**, 1074-1075.

5. Lofgren, C.S. 1986. In *"Economic Impact and Control of Social Insects"* (S.B. Vinson, Ed.), pp. 227-256. Praeger, NY.

6. Oi, D.H., and Williams, D.F. 2002. *J. Econ. Entomol.* **95**, 558-562.

7. Porter, S.D., Fowler, H.G., and MacKay, W.P. 1992. *J. Econ. Entomol.* **85**, 1154-1161.

8. Snowden, K.F., Logan, K.S., and Vinson, S.B. 2002. *J. Eukaryot. Microbiol.* **49**, 447-448.

Table 1. Patterns of *T. solenopsae* infections in *S. invicta* population in 2002.

Sites	Total number of plots	Number of plots sampled	Number of plots analyzed	Number and percent of infected plots
Adam's Ranch, Bryan County	19	16	14	12 (85.7%)
Bowles' Ranch, Bryan County	22	19	19	17 (89.5%)
McCoy's Ranch, McCurtain County	20	19	14	0

Table 2. Patterns of *T. solenopsae* infections in *S. invicta* population in 2003.

Sites	Total number of plots	Number of plots sampled		Number of plots analyzed		Number and percent of infected plots	
		Spring	Fall	Spring	Fall	Spring	Fall
Adam's Ranch, Bryan County	19	17	17	12	17	3 (30%)	12 (70.6%)
Bowles' Ranch, Bryan County	22	19	17	15	17	5 (33.3%)	10 (58.8%)
McCoy's Ranch, McCurtain County	20	17	20	14	20	0	0

Fig 1. Photomicrograph of binucleate free spores detected from RIFA samples

a. 400X magnification

b. 1000X magnification
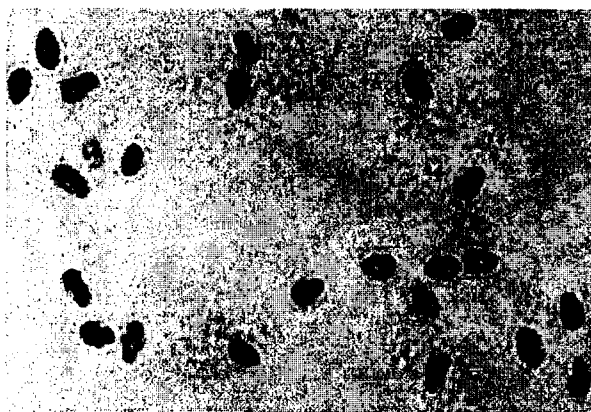
A New Bioassay for Evaluating Fire Ant Repellants

Jian Chen

Biological Control of Pests Research Unit, USDA-ARS, 141 Experiment Station Road,

Stoneville, MS 38776

Abstract

A new bioassay for evaluating fire ant repellants was developed. The active ingredient was incorporated into sand within a liquid scintillation vial with an entry hole on the cap. Through the entry hole, fire ants dug and removed sand from the vial. The differences in amount of sand removed from the treated and control vials were used to evaluate chemical repellency. By using this bioassay, dimethyl and diethyl phthalates were found to be strong repellants to red imported fire ants. Two-choice tests showed that the minimum repellent concentration within 24 h was 100-ppm for both dimethyl and diethyl phthalates.

Introduction

Red imported fire ant, *Solenopsis invicta* Buren, is one of the most important medical and agricultural pest ants in the United States. Tremendous effort has been made on developing

---

control measures for the red imported fire ant. It has resulted in numerous insecticide products (Williams et al. 2001). With the increasing concern of detrimental effect of insecticides on the environment, non-toxic or less-toxic measures are always desirable. Using fire ant repellants is an alternative for some special situations, such as hospital, plant nursery, and electric circuitry (Vander Meer et al. 1994). A number of materials have been reported as fire ant repellants (Blum et al. 1991; Kaakeh and Dutch 1992; Vander Meer et al. 1994, 1996, 1998; Oi and Williams 1996; Drees et al. 2000; Anderson et al. 2002). Different bioassays have been used in evaluating fire ant repellants. By using a Y-tube olfactometer, Vander Meer et al. (1996, 1998) found a number of fire ant repellants. By comparing the number of ants on a filter paper treated with a leachate solution to that of ants on a control in a plastic tray, Anderson et al. (2002) found that sage (*Saliva* sp.), pine needle, and cedar shaving water suspension were repellent to red imported fire ant colonies. Oi and Williams (1996) found bifenthrin and tefluthrin in potting soil repelled red imported fire ants.

Red imported fire ants build mounds and underground foraging tunnels by digging up surrounding soil (Markin et al. 1975). In the laboratory, fire ants show digging behavior whenever adequate substrate is provided. The objective of this study is to develop an easy-to-perform repellency bioassay based on fire ant digging behavior. Bioassay arena, apparatus, and experimental designs are addressed. Results of the repellency test on nine phthalates are presented.

Materials and Methods

Bioassay Arena and Apparatus. The bioassay arena was a 33.5 by 6.5 cm round aluminum pan with no lid. The inner side of the pan was coated with Fluon to prevent ants from

escaping. The bioassay apparatus was a capped Wheaton liquid scintillation vial (2.8 cm by 6.1 cm). At the center of the cap was a 3-mm diameter entry hole. Each vial was placed on a metal screen which covered a 6.0 by 1.5 cm Petri dish (Fig.1). In this way, most sand removed by ants was confined in the Petri dish. All vials, with Petri dishes on the bottom, were placed around another 10 by 1.5 cm Petri dish (center container), which was co-centered with the test arena, so that all vials were equally accessible to fire ants, which were released in the center container (Fig. 2).

Multiple-choice Test on Nine Phthalates Sand was washed with distilled water and dried at 350 °C. A 15 ml acetone solution of test chemical was mixed with 250g sand in an aluminum pan. After acetone evaporated (10 minutes), 16 ml distilled water was added and mixed with sand. An average 36.80 g (± 0.8g) sand was added into each vial with a concentration of 200-ppm for each test chemical. Sand in the control vial was treated only with acetone. Dimethyl, diethyl, dipropyl, dibutyl, dipentyl, dihexyl, diheptyl, dioctyl, and dinonyl phthalates were tested. Locations of vials were randomized. One thousand fire ant workers were introduced into the center container in each arena. The experiment was conducted at 25 °C. After 24 h, sand in each vial was collected, dried at 350 °C for one hour, and weighed. Two colonies, colony #1 and colony #2, are used for this experiment. The experiment was replicated 5 times for each colony. The general linear model analysis of variance and LSD test (PROC GLM; SAS Institute 1999) was used to compare the amount of sand removed by ants among treatments. Significance was determined at $P < 0.05$.

Multiple-choice Test on Five Phthalates The purpose of this experiment was to check whether reducing the number of chemicals in one test would further separate test chemicals statistically. The experimental design and statistical analysis were the same as noted previously

except the number of choices was reduced to six: dimethyl, diethyl, diheptyl, dihexyl, and dioctyl phthalates and control. These chemicals were not statistically separated in the experiment with 9 phthalates. Six hundred fire ants were used for each test arena. Colonies are the same as those used in the previous test.

Two-choice Test on Dimethyl and Diethyl Phthalates The bioassay arena, apparatus, and procedure were similar as those described in the multiple-choice test, except only two choices, treatment and control, were presented in the arena. One vial was treated with either dimethyl or diethyl phthalate, and the other with only acetone. Sand moisture was adjusted to 6%. Three hundred fire ants were used for each test arena. Concentrations of 25, 50, 100-ppm were used. For each concentration, there were 5 replications. The experiment was conducted at the temperature of 25 °C. Paired t-test (critical P-value = 0.05) was used to compare mean amount of sand removed in treated vial with that in control vial for each concentration. Two colonies, colony #3 and colony #4, were used for this experiment.

Results

Multiple-choice Test on Nine Phthalates There was a significant effect of test phthalates on the amount of sand removed from vials for colony #1 ($F = 3.12$, $df = 9$; $P = 0.007$) and colony #2 ($F = 2.30$, $df = 9$; $P = 0.04$) (Table 1). Although dimethyl and diethyl phthalates were numerically most repellent to fire ants, few statistical separations were found. For colony #1, dimethyl and diethyl phthalates were statistically separated from dibutyl, dipropyl, dipentyl phthalates and control. Dibutyl, dipentyl, dinonyl phthalate and control were separated from dimethyl and diethyl phthalates for the colony #2. Significant differences among test arenas

were found for colony #1 ($F = 2.67$, $df = 4$; $P = 0.048$) and colony #2 ($F = 10.28$, $df = 4$; $P < 0.0001$).

Multiple-choice Test on Five Phthalates The effect of the tested phthalates on the amount of sand removed was significant for both colony #1 ($F = 21.19$, $df = 5$; $P < 0.0001$) and colony #2 ($F = 5.57$, $df = 5$; $P = 0.002$) (Table 2). With six choices in the test arena, dimethyl and diethyl phthalates were statistically separated from the rest of test chemicals for colony #1. For colony #2, dihexyl and dioctyl phthalates were successfully separated from dimethyl and diethyl phthalates. No significant difference among test arenas were found for both colony #1 ($F = 1.48$, $df = 4$; $P = 0.25$) and colony #2 ($F = 0.20$, $df = 4$; $P = 0.94$).

Two-choice Test on Dimethyl and Diethyl Phthalates The results are summarized in Fig. 3. At 100-ppm level, there were significant differences between controls and treatments for dimethyl and diethyl phthalates. Below 100-ppm level, the difference between control and treatment was not significant.

Discussion

The red imported fire ant is a mound-building species. Mound and extensive underground foraging tunnel system, which are constructed by digging surrounding soil, are essential to the success of red imported fire ants. Hubbard (1974) found fire ants were able to recognize soil from their own nest and dug in it preferentially. The results of the present study demonstrated that ant digging behavior was affected by the chemical nature of the substrate; such effect can be quantified by using the weight of the excavated substrate.

With an adequate number of test chemicals in the arena, multiple-choice test can be used to screen multiple chemicals in a single test. For the multiple-choice test to be sensitive, the

number of choices in a test must be carefully controlled. For example, with nine chemicals in the multiple-choice test, dioctyl phthalate could not be separated from dimethyl and diethyl phthalates; however, when the number of test chemicals was reduced to five, such separation was achieved.

Choice tests have been used in fire ant repellency tests, such as the Y-tube olfactometer method (Vander Meer et al. 1996, 1998). Y-tube olfactometer was designed for testing volatile compounds. However, not all ant repellants work through the olfactory receptors. Greater repellency may be archived by combining chemicals with different sensory input modes (Storey et al. 1996). This new method can be used to test repellency through both olfactory and gustatory receptors.

Comparing number of ants on treated and control objects is common in fire ant repellency tests. It is technically difficult to count ants on an object without immobilizing them, particularly when there are a great number of ants on the object. This digging bioassay provides a very easy method to quantify the repellent effect – weighing the excavated sand.

Dimethyl and diethyl phthalate are common ingredients of mosquito repellents (Schreck 1991; Frances et al. 1993; Anonymous 1995; Frances and Cooper 2002). It was reported that diethyl phthalate was also used as ingredient in 67 cosmetic formulations at concentration up to 50% (Anonymous 1995). The results of this study showed that dimethyl phthalate and diethyl phthalates are also repellants to red imported fire ants. Both dimethyl and diethyl phthalates are inexpensive chemicals (dimethyl phthalate: $15.30 per liter; diethyl phthalate: $17.00 per liter, Fisher Chemicals, Fairlawn, NJ). They may be good candidates to be used in developing fire ant repellant formulations.

References Cited

Anonymous 1995. Toxicological profile for diethyl phthalate. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substance and Disease Registry. PB/95/264214/AS

Anderson, J. T., G. H. Thorvilson and S. A. Russell. 2002. Landscape materials as repellents of red imported fire ants. Southwest. Entomol. 26: 195-203

Blum, M. S., D. M. Everett, T. M. Jones and H. M. Fales. 1991. Arthropod natural products as insect repellents. pp. 14-26. *In* P. A. Hedin [ed.], Naturally occurring pest bioregulators. American Symposium Circular 449, Washington, DC

Drees, B. M, J. A. Reinert and S. B. Vinson. 2000. Red imported fire ants may find some landscape design elements unattractive (fireant.tamu.edu/materials/index.html)

Frances S. P. and R. D. Cooper. 2002. Personal protection measures against mosquitoes A brief history and current use of repellents by the Australian Defence Force. ADF Health 3: 58-63

Frances S. P., Eikarat N., Sripongsai B. and C. Eamsila. 1993. Response of *Anopheles dirus* and *Aedes albopictus* to repellents in the laboratory. J Am. Mosq. Control Assoc. 9: 474-4766

Hubbard, M. D. 1974. Influence of nest material and colony odor on digging in the ant *Solenopsis invicta* (Hymenoptera: Formicidae). J. Georgia Entomol. Soc. 9: 127-132

Kaakeh, W. and J. D. Dutch. 1992. Foraging preference of red imported fire ants (Hymenoptera: Formicidae) among three species of summer cover crops and their extracts. J. Econ. Entomol. 85: 389-394

Markin, G. P., J. O'Neil and J. Dillier. 1975. Foraging tunnels of the red imported fire ant,

Solenopsis invicta (Hymenoptera: Formicidae). J. Kan. Entomol. Soc. 48: 83-89.

Oi, D. H. and D. F. Williams. 1996. Toxicity and repellency of potting soil treated

with bifenthrin and tefluthrin to red imported fire ants (Hymenoptera: Formicidae). J.

Econ. Entomol. 89: 1526-1530

SAS Institute, 1999. SAS/STAT user's guide version 8. SAS Institute, Cary, NC

Schreck C. E. 1991. Permethrin and dimethyl phthalate as tent fabric treatments against *Aedes*

aegypti. J Am. Mosq. Control. Assoc. 7: 533-535

Storey, H. H., L. K. Gaston, R. G. Gerber, C. B. Sisk and P. A. Phillips. 1996.

Formulating farnesol and other ant-repellent semiochemicals for exclusion of argentine

ants (Hymenoptera: Formicidae) from citrus trees. Environ. Entomol. 25: 114-119

Vander Meer, R. K., J. A. Seawright, and W. A. Banks. 1994. The use of repellents

for area exclusion of pest ants. p. 494. *In* Wildey, K. B., and W. H. Robinson [eds],

Procedings of the First International Conference on Insect Pests in the Urban

Environment. St John's College, Cambridge, 30 June – 3 July 1993

Vander Meer, R. K., W. A. Banks and C. S. Lofgren 1996. Repellents for ants. United

States Patent. Date: December 24, 1996, Number 5,587,401

Vander Meer, R. K., W. A. Banks and C. S. Lofgren 1998. Repellents for ants. United

States Patent. Date: February 24, 1998, Number 5,721,274

Williams, D. F., H. L. Collins and D. H. Oi. 2001. The red imported fire ant (Hymenoptera:

Formicidae): An historical perspective of treatment programs and the development of

chemical baits for control. Amer. Entomol. 47: 146-159
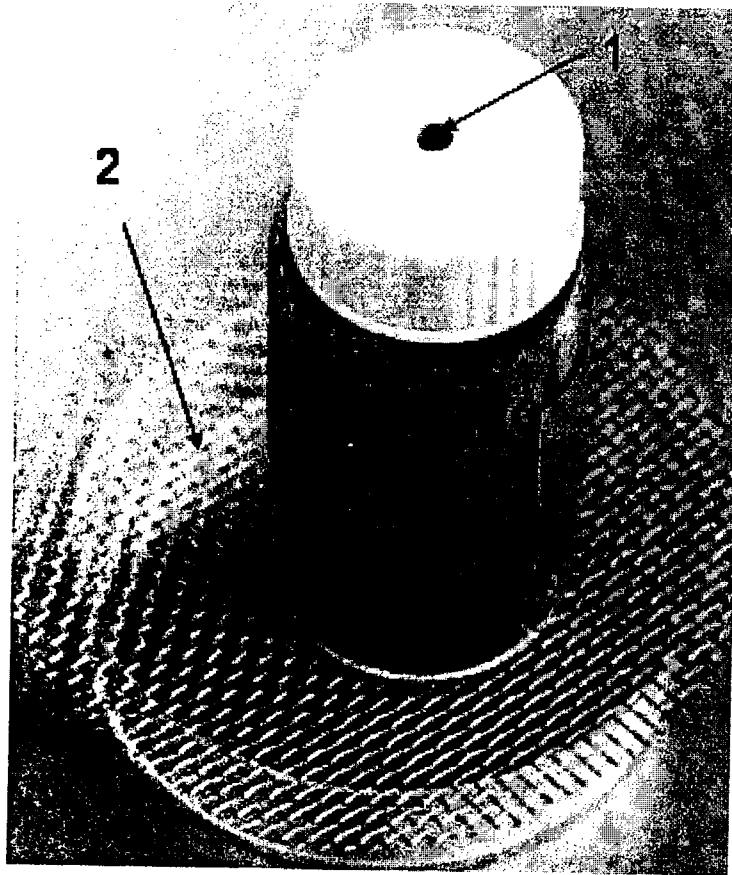
Figure 1. The bioassay apparatus showing (1) entry hole on the cap and (2) metal screen on the top of a Petri dish.
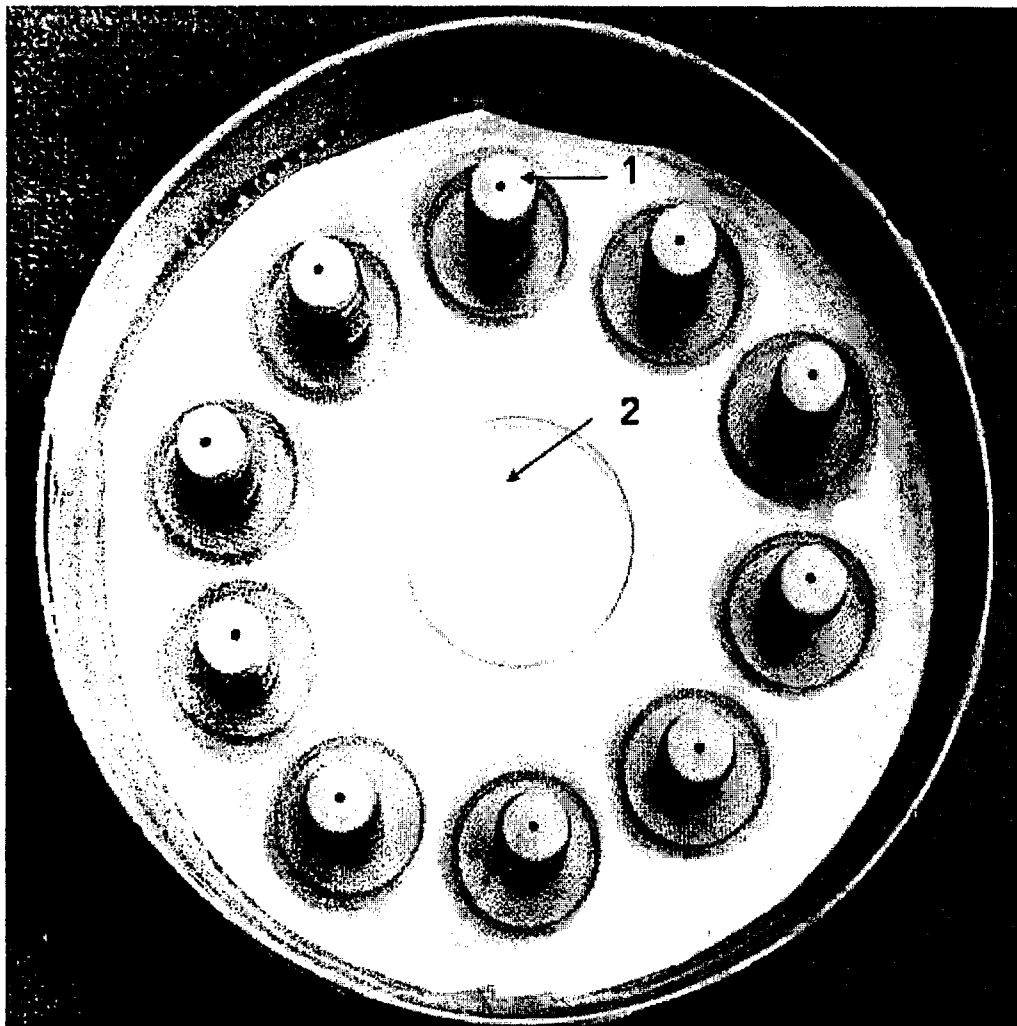
Figure 2. Test bioassay arena showing (1) apparatus and (2) center container.

Table 1. Repellency of nine phthalates against two colonies of *S. invicta*, based on mean amount of sand removed by ants 24 h after release. There were five replicates for each colony.

Colony	Chemical	Mean amount of sand removed (g ± SE)*
#1	Dibutyl phthalate	1.67 ± 0.51 a
	Dipropyl phthalate	0.70 ± 0.29 ab
	Control	0.89 ± 0.17 ab
	Dipentyl phthalate	0.86 ± 0.22 b
	Diheptyl phthalate	0.73 ± 0.30 bc
	Dinonyl phthalate	0.67 ± 0.22 bc
	Dihexyl phthalate	0.66 ± 0.56 bc
	Dioctyl phthalate	0.31 ± 0.14 bc
	Diethyl phthalate	0.01 ± 0.01 c
	Dimethyl phthalate	0.00 ± 0.02 c
#2	Dibutyl phthalate	1.68 ± 0.89 a
	Dipentyl phthalate	1.58 ± 0.73 ab
	Control	1.32 ± 0.45 ab
	Dinonyl phthalate	1.07 ± 0.62 b
	Dipropyl phthalate	0.97 ± 0.43 bc
	Dioctyl phthalate	0.67 ± 0.36 bc
	Dihexyl phthalate	0.65 ± 0.25 bc
	Diheptyl phthalate	0.47 ± 0.39 bc
	Diethyl phthalate	0.29 ± 0.24 c
	Dimethyl phthalate	0.04 ± 0.01 c

*: Means followed by the same letter are not significant different ($P = 0.05$; LSD test).

Effect of phthalates was significant for both colony #1 ($F = 3.12$, $df = 9$; $P = 0.007$) and colony #2 ($F = 2.30$, $df = 9$; $P = 0.04$). Difference among arenas was significant for both colony #1 ($F = 2.67$, $df = 4$; $P = 0.048$) and colony # 2 ($F = 10.28$, $df = 4$; $P < 0.0001$).

Table 2. Repellency of five phthalates against two colonies of *S. invicta*, based on mean amount of sand removed by ants 24 h after release. There were five replicates for each colony.

Colony	Chemical	Mean amount of sand removed (g ± SE)*
	Dioctyl phthalate	0.66 ± 0.08a
	Dihexyl phthalate	0.31 ± 0.08b
	Diheptyl phthalate	0.25 ± 0.07b
#1	Control	0.17 ± 0.03b
	Diethyl phthalate	0.01 ± 0.02c
	Dimethyl phthalate	0.01 ± 0.02c
	Control	1.27 ± 0.45a
	Dihexyl phthalate	0.98 ± 0.16a
	Dioctyl phthalate	0.80 ± 0.18ab
#2	Diheptyl phthalate	0.30 ± 0.06bc
	Dimethyl phthalate	0.01 ± 0.003c
	Diethyl phthalate	0.007± 0.003c

*: Means followed by the same letter are not significant different ($P = 0.05$; LSD test).

Effect of phthalates was significant for both colony #1 ($F = 21.19$, $df = 5$; $P < 0.0001$) and colony #2 ($F = 5.57$, $df = 5$; $P = 0.002$). Difference among arenas was not significant for both colony #1 ($F = 1.48$, $df = 4$; $P = 0.25$) and colony # 2 ($F = 0.20$, $df = 4$; $P = 0.94$).
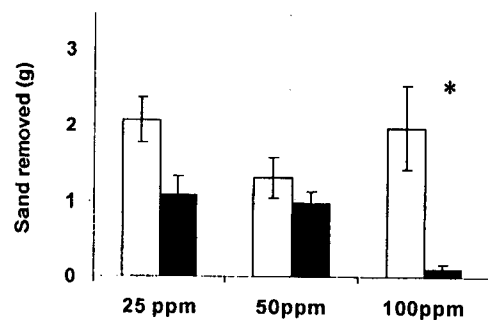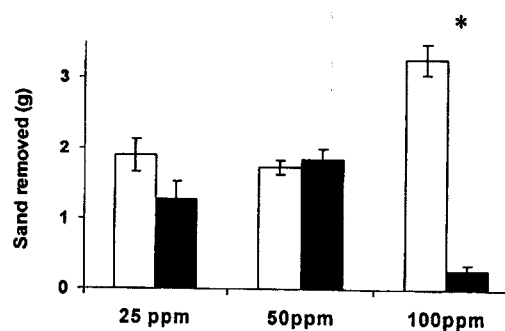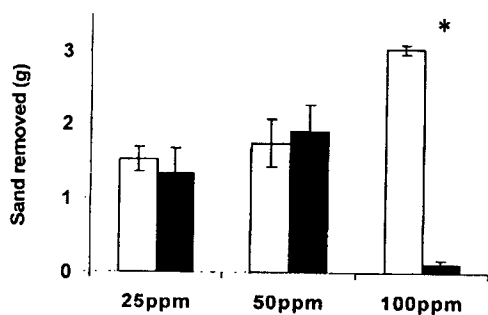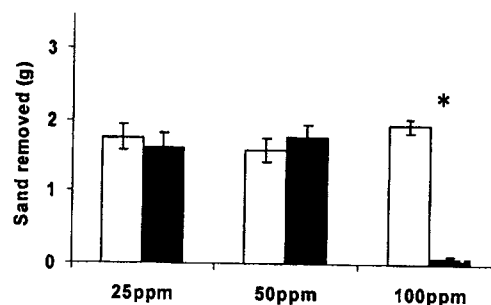
Figure 3. Amount of sand removed (mean ± SE) from treatment and control vials in a series of two-choice repellency tests on dimethyl and diethyl phthalates against *S. invicta*. Concentration with an asterisk indicates significant difference between treatment and control (Paired t-test).

Some methods to rear small special colonies of fire ants for special reasons.

S. Bradleigh Vinson, Sherry Ellison and M. Bashir

Texas A&M University, College Station, TX. 77843

I will discuss two questions.

1. How to rear ants for special needs such as producing fertile males from virgin females or rearing sterile males or workers from genetically modified females.

Wc wanted to develop a method to rear large numbers of males for genetic studies. Although rearing a few males is not a problem, we wanted a large number and we could not find any literature regarding the rearing of male fire ants. The problem was not as obvious as it first appeared. Using a monogyne colony was not viable option, as such a colony could not be sustained. Using a polygyne colony that consisted of a queen and a number of virgin females appeared to be an option, but there were several problems. One was a concern that a high number of virgin females and a queen would lead to the elimination of some of the virgins. However, of greater concern was the quality of the males. The reason is that a polygyne fire ant colony consists of brood, workers, males and several types of reproductive females. These females are 1) fertile "Queens" that produce workers, alate females and males, 2) dealate virgin females that produce only males, and 3) females that produce sterile males. If a colony is set up with a number of queens and a number of virgin alates there is a good possibility that sterile males would be produced that would be a problem and we would have a challenge identifying the quees producing the sterile males. Secondly, we would not have a single genetic pattern in the males produced. If we set up with a single female as described as #1 above, a normal colony may result, although it will require a few workers if an older established queen is used. If females described as # 2 are used it is unlikely that these females can rear the males to the adult stage with out some workers, but such a colony is not sustainable. If sterile male producing females are used, #3, a few sterile males would be produced, if some workers were added. However, as noted for #2 even with a few workers added to the colony the colony would not be sustainable.

We tried a number of approaches and found the following method to be effective. We first collect a large field polygyne colony with a large number of alate (virgin) females. The colony is removed from the soil (See Jouvenaz et al 1977. Fla. Entomol. 60: 275-279) placed in a large plastic shoe or sweeter box (see Chen and Vinson, 1999. Annals Entomol. Soc. Am. 92:578-586). This is referred to as the parent colony. We then remove around 4 to 500 workers to a new nest box to form a queenless sub-colony. Next an alate female from the parent colony is added. By using a lot of workers enough resources are moved from the parent colony to build the reproductive competence of the "queen". As

workers die new workers or worker pupae from the parent colony are added every few weeks. Using this technique we have been able to maintain colonies that only produce fertile males (Table 1). The technique could be used to maintain any special queen, such as a transformed or genetically altered queen, or a queen that produces sterile males.

Number of parent colonies	Number of satellite colonies	Number of males produced/ colony/each 2 month period		
		2 months	4 months	6 months
4 polygyne	7	30 - 53	113 - 163	170 - 251
2 monogyne	3	30 - 45	105 - 137	131 - 210

Table 1:Male production on a 2 month basis in satellite colonies set up as described.

2. We also wanted to improve our rearing of new colonies starting with inseminated females collected after a mating flight. We have generally had a success rate of around 30% by placing 2 flight queens / 5 ml cotton plugged test tube that was kept moist. However, we wanted to see if we could improve the survival of the queens and get healthier colonies in a shorter time. One approach was to add workers, but we opted to use worker pupae because they would be expected to be more readily accepted by the queen and less of a chance of the workers attacking the queen. Although this research is ongoing, we have some results that were not expected. We began by setting up test tubes (replicated 10 times each) with the following.

A. 1 flight queen / test tube.
B. 2 flight queens / test tube.
C. 1 flight queen + 25 white worker pupae from a polygyne colony added the day of set up / test tube.
D. 1 flight queen + 25 white worker pupae from a polygyne colony added 7 days after set up / test tube.
E. 1 flight queen + 25 white worker pupae from a polygyne colony added 14 days after set up / test tube.

The results in table 2 show the number of eggs, larvae, pupae and adult workers at the end of two months. Starting with more than 1 queen resulted in more eggs, larvae, pupae and workers after 2 months than starting with one queen. However, the survival of the queens when starting with more than one was low. Although preliminary, these results suggest that starting with several queens is a waist of queens. In contrast, the addition of pupae did not improve queen survival. These results suggest that some queens are just not healthy. The addition of pupae was not helpful or negative in regard to reproduction by the queen when added as the newly mated queens were set up. However, the addition of pupae 14 days after the queens were set up appeared to stimulate the reproductive effort on the part of the queens. As a result the addition of pupae 14 days after the founding colonies are set up appears to result in larger colonies. It was surprising that the addition of pupae at the start of the queens colony foundation was not a benefit the queen. It

appears that the reproductive effort of the newly mated queen requires some period of maturation before becoming fully functional.

We are in the process of expanding this study and hopefully will have additional data next year.

Treatment	% colony survival	% queen survival	Ave. # of eggs	Ave. # of larvae	Ave. # of pupae	Ave. # of workers
1 Q	40	40	85.7	184.7	157.0	100.7
3 Q's	50	16.6	112.4	242.8	202.8	183.2
Q + pupae day 0	40	40	88.5	199.7	191.5	139.0
Q + pupae day 7	50	50	83.2	229.4	178.0	149.2
Q + pupae day 14	50	50	114.2	290.2	240.6	216.4

Table 2: Survival of newly mated queen collected following a mating flight and allowed to found colonies with help from another queen or workers.

Area-wide Suppression Project

Areawide Fire Ant Suppression in Florida

Roberto M. Pereira

USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd
Drive, Gainesville, FL 32608

The USDA-ARS **Areawide** Fire Ant Suppression project aims to demonstrate the use of a biologically based integrated pest management (IPM) strategy for long-term suppression of fire ants in the U.S. pastures. Natural enemies of fire ants, such as parasitic decapitating flies and a microsporidian pathogen, are used in conjunction with chemical bait applications (hydramethylnon **+** methoprene). The project is conducted in Florida, Oklahoma, Texas, and South Carolina (all infested with the red imported fire ant, *Solenopsis invicta),* and in Mississippi, where populations of the black imported fire ant *(Solenopsis richteri)* and the **red/black** hybrid are targeted. In all states, IPM sites **are** compared with sites where only chemical baits are applied but no biological controls are established.

Chemical baits have been applied to the Florida sites four times over the last two years, and the fire ant populations have been maintained at low levels in the treated areas. During this period, no significant detrimental effect on the native ant population has been detected. However, beneficial effects of controlling the fire ant population cannot yet be detected in the native fauna.

Release of decapitating flies is an important element of the project on **areawide** suppression of fire ants in pastures. The decapitating flies *Pseudacteon tricuspis* had been established in Florida before the initiation of the project. A new biotype of *Pseudacteon curvatus* was released and established in the project site in Florida, and continues to expand its range in the area. This biotype was obtained from *S. invicta* populations in Argentina as opposed to a previous *P. curvatus* biotype (established in Mississippi) that was collected from *S. richteri* populations. A third decapitating fly species, *Pseudacteon litoralis,* has been released but not **confirmed** established in the **areawide** site in Florida. The fire ant disease caused by *Thelohania solenopsae* was also established in Florida before initiation of the **areawide** project. In areas treated with the chemical baits, the prevalence of the disease has decreased with a decrease in fire ant populations.

A project **website** (http://www.ars.usda.gov/fireant/) has been updated and includes educational videos describing the fire ant disease caused by *T. solenopsae* and the decapitating flies. These videos are also included in a new educational CD prepared recently for public distribution.

Phorid Fly Range Expansion and Mound Suppression in a Heavily Infested Area

Charles L. Barr
Alejandro A. Calixto

Texas Cooperative Extension

In 2001 the USDA-ARS approved a five year demonstration of the effects of biological control organisms in combination with broadcast baits for the long term suppression of fire ants (*Solenopsis invicta* Buren) in pastures. The basic concept was that the baits would cause an initial ltnock-down of high fire ant populations and the biocontrol organisms would then delay or even prevent reinvasion of the treated areas, thus lengthening the time between the expensive bait treatments.

Materials and Methods

Two working ranches, about 25 miles apart, were chosen for Texas' part of the multi-state effort. Five Eagle Ranch, in Bwleson County, was chosen as the "biological control" site and the NK Cattle Co., in Brazos County, was chosen as the "untreated" site. Each site has 50, 118-acre monitoring plots scattered around it. Twenty plots are located within 300 acre blocks that are treated with bait to maintain 90% control. The remaining 30 plots surround the treated blocks. Therefore, the experimental design consists of four treatments: At NK Cattle Co., 1) untreated, no biological control and 2) bait-treated, no biological control; at Five Eagle Ranch, 3) untreated, biological control and 4) bait-treated, biological control.

The first phorid flies (Pseudacteon tricuspis) were released at Five Eagle Ranch in April, 2002. During the same period, monitoring plots were established and initial evaluations were conducted both there and at NK Cattle Company. Semi-annual evaluations include: counts and ratings of active mounds, pitfall traps (8 per plot), hot dog foraging sampling (10 per plot) and sampling of the other biocontrol organism, Thelohania solenopsae. Broadcast baits were applied to the treated areas by air on May 30, 2002 and again on October 11.

Periodic searches for phorid flies were conducted around the release area of Five Eagle Ranch through the summer and fall of 2002. Detection efforts began again in the spring of 2003, followed by a second release of flies beginning April 25, 2003. The second release was of the "Formosan biotype" of P. *tricuspis*, originally collected from an area of South America more similar to the climate of Central Texas than the flies of the first release. All flies were supplied by Dr. Sanford Porter, USDA-ARS, CMAVE, Gainesville, Florida. By the fall of 2003, phorid flies were becoming very abundant around the immediate release site. We gradually widened our detection area until, on November 11, 2003, detection efforts were carried out to the north, south and west boundaries of the property. All detection efforts in 2003 were conducted using a modified electric livestock prod.

The statistical analysis was conducted by dividing the plots of Five Eagle Ranch into four groups: 1) untreated, infested (by phorid flies); 2) untreated, uninfested; 3) bait-treated, infested and; 4) bait-trcated, uninfested. Because the infestation grew in both extent and fly population, some arbitrary parameters had to be established to group the plots for analysis. Plots in the area

where flies were detected during the summer and had five or more flies present over a mound in September were considered "infested." Those in areas where no flies were detected during the summer were considered "uninfested." To help prevent bias in plot designation, the plots were grouped before mound count data had even been summarized.

Statistical analyses were performed using PC SAS general linear model procedure due to the unbalanced data set and t-test procedures, $P < 0.05$. Percent data were analyzed using arcsine transformation, but figures display percent of pre-count means.

Results and Discussion
Phorid Range Expansion

Our first successful recovery of a phorid fly was made in July, 2002 when three flies were found during an afternoon of looking - probably second generation offspring. After this time, no flies were recovered for the rest of the year. Consequently, the Formosan biotype release was scheduled for late April. A few days before the release began, three flies were detected in a wooded area in the release site. These had obviously overwintered and were a strong indication of a successful establishment. Throughout the summer, an increasing number of flies were detected more quickly and in greater numbers across the release site, eventually spreading west down a creek bottom. During the fall evaluations, one or two flies per mound were detected in and across the half-mile-wide the bait-treated area from the release site.
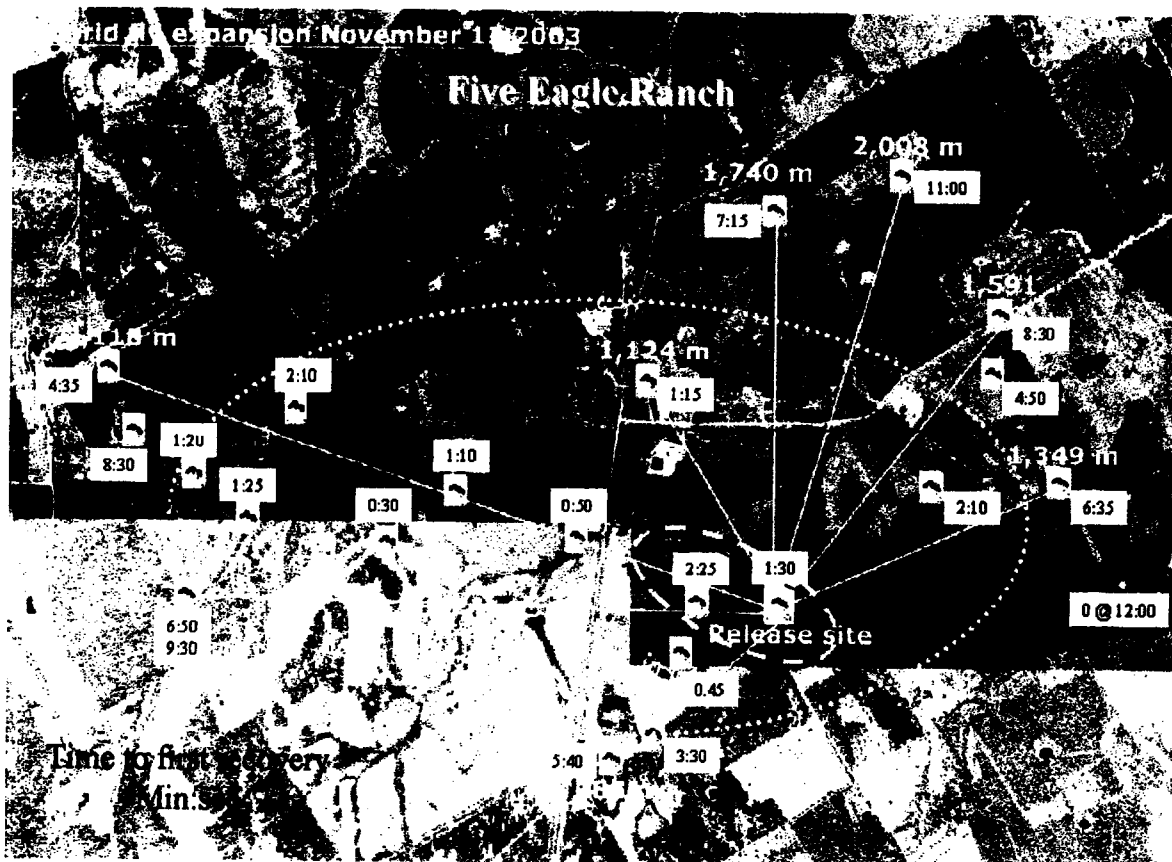
Results from phorid detection efforts on November 11, 2003 showed that the flies had expanded their range at least three km to the west and over one km in every other direction. (See Fig. 1). It was likely that the flies had spread farther, but we were limited by the Five Eagle Ranch property boundaries. The dotted oval in Fig. 1 indicates first detection times of less than two and a half minutes. Within the release area, it was common to detect 10 or more flies over a mound upon disturbance.

Analysis of Mound Count Data

Over the course of 2003, we felt that the number of visible fire ant colonies in the area of the highest phorid fly populations were dropping. Though mound counts and ratings had been collected for all 50 plots since May 2002, none of these data had been analyzed or summarized in any way. Therefore, grouping of the plots into the four categories was done based strictly on observation of phorid fly extent and population and not on any numerical data on mound counts. In doing this, we reduced the chances of bias in grouping the plots, albeit inadvertently.

The first analysis was done using SAS, PROC GLM to account for the unbalanced data set (n=5, 10, 15 and 20). The mean number of active mounds pre infestation (May 2002) and post-infestation (September 2003) were analyzed within each evaluation date. As shown in Fig. 2, the plots that would fall into the untreated, infested area (n=10) had significantly ($P < 0.05$) more mounds than any of the other plots - a mean of 43.6 mounds per 1/8-acre circle vs 25.7 in the other three groups. Figure 2 also shows how the mean of those untreated, infested plots dropped to roughly the same level as the untreated, uninfested plots by fall 2003. In the treated area, pre-treatment means were almost identical between the two groups, but the fly-infested plots dropped below the mean of the uninfested plots.

Though trends are evident, it is not appropriate to compare all four groups to each based strictly on phorid fly presence since plots in the treated area had been treated with broadcast bait twice and their fire ant populations reduced to near zero, in most cases. To more fairly compare the possible effects of the phorid flies and account for pre-infestation differences, it was more appropriate to analyze the differences between pre and post-infestation mound numbers in the same plots using percent control (of pre-count) as the dependent variable [1 - (post-count/pre-count)]. Most importantly, data for the treated and untreated plots were analyzed separately to account for the effects of the bait treatments. Given only two treatments and percent data, the analyses were conducted using student's t-test ($P < 0.05$) and arcsine transformation of the calculated percent control.

Figure 3 shows the results of the comparison of untreated plots, pre versus post-infestation. Plots in the infested area had a mean 67.2% control compared to only 35.1% in uninfested plots. With a p-value of 0.0001, this difference is highly significant. Figure 4 shows the same analysis for bait-treated plots. Differences were neither as dramatic as in the untreated plots nor statistically different ($P < 0.05$): infested plots had 85.2% percent control vs 72.6% for uninfested, $P = 0.1867$. Nevertheless, a trend of more control in fly-infested plots was still indicated.

2003 Update on the Areawide Fire Ant Management Program in Oklahoma

Russell Wright[1], Vedham Karpakakunjaram[1] and Wayne Smith2
[1]Department of Entomology & Plant Pathology, Oklahoma State University
Stillwater, OK 74078
'Oklahoma Cooperative Extension Service, 1901 S. 9th Street, Durant, OK 74702

Abstract

Three 150 acre test sites were established for the Oklahoma part of the USDA-ARS Areawide Suppression of Fire Ants Program in 2002. The two sites in Bryan County, the Adams Ranch and the Bowles Farm, about 30 miles apart, constitute the biological control sites where treatments include the use of baits and the release of the parasitic fly Pseudacfeon *tricuspis* and the establishment of the pathogen Thelohania solenopsae. The third site, the McCoy farm, is in McCurtain County 120 miles east of Bryan County, serves as the non-biological control site, where only fire ant bait applications are used for control of fire ants.

An evaluation of all plots in the treatment sites from May 20-22, 2003, indicated the number of red imported fire ant mounds exceeded the treatment threshold, 2.5 mounds per plot, at the Adams and Bowles sites in Bryan County but not at the McCoy site in McCurtain County. An aerial application of 0.75 lb. of EXTINGUISH (methoprene) bait and 0.75 lb. of AMDRO PRO (hydramethylnon) bait per acre was made at the Adams and Bowles site on June 11, 2003. A 30 day post treatment evaluation indicated that the number of fire ant mounds at all sites were below the threshold level. A 90 day post treatment evaluation made in mid-September at all sites indicated that the fire ant mounds exceeded the treatment threshold at the Adams and McCoy sites. These sites were treated with an aerial application of the recommended rates of the baits on October 21 and 22 respectively. A partial evaluation of five plots at each site made on December 4 and 5, 45 days post-treatment, indicated poor reduction of the fire ant mound density with the October treatment. Follow up evaluations will be made in May 2004. Fewer fire ant mounds were detected in the treated and untreated areas at all three sites in the summer and fall of 2003. This reduction was likely due to the extremely high summer temperatures and a prolonged lack of rainfall.

Releases of the parasitic fly, Pseudacfeon *tricuspis*, were made at the Adams and Bowles sites during 2002. Populations were established at both sites and were present at both sites in October 2002. However, no flies were found at either site in the spring of 2003. Evidently these populations did not over winter successfully. Subsequent releases of *P. tricuspis* were made at both sites in June and August 2003, but no subsequent generations were found. The microsporidian Thelohania solenopsae was found in 85% of the mounds sampled at the Adams and Bowles sites in 2002. None were found in fire ant populations at the McCoy site. The number of fire ant mounds infected with *T.* solenopsae in the spring of 2003 was 30% and 26% at the Adams and Bowles site respectively, but the infection rate had increased to 65% and 47% respectively by September 2003. We have no explanation for the apparent reduction in Thelohania infection rates in 2003 from 2002. This pathogen was not detected in fire ant mounds at the McCoy site in either the spring or fall 2003 samples.

Other

ECOLOGICALLY BENIGN METHOD FOR FIRE ANT CONTROL

By
R. E. Driscoll, Sr., Hugh M. Ettinger, M. Dale Mayes

Synopsis: This is the story of the provision of an incentive to do something and a history of what was done to solve a specific problem. The product being discussed is produced with materials that are listed in U. S. EPA Bulletin PR 2000-6, Minimum Risk Insecticides. The products used in this insecticide are truly organic in nature and are all biodegradable.

PROVISION OF INCENTIVE TO DO SOMETHING:

The incentive to solve the problem was provided by the author's wife some ten years back.

We reside in Parker County near the city of Weatherford, Texas. This is a rather central location in the county some 30 miles west of the City of Fort Worth. We have lived at this location since 1978. The property in question is a plot of land approximately 2.5 acres in size. It is a rather nice piece of land and at the time of our original occupancy had a nice population of squirrels, quail, rabbits in due season, frogs (or toads if you will) and a rather minor population of rattle snakes. (Which my wife did not encourage!)

One afternoon my wife came in from doing some yard work and told me that she had been bitten several times by some type of an insect. While it was initially not realized what was happening it was later found out that we had just been introduced to the fire ant.

The original area where she was working was sprayed the with Malathion thinking that her problem was in all likelihood a spider or some such creature..

Chemical treatments are no longer used.

Over the next 24 hours the spots where she had been 'bitten' or 'stung' developed a redness and a small pustule was formed. We opened the pustules and treated them with an antiseptic solution and all seemed to be well. This was a dilute Clorox Bleach Solution.

THE DEVELOPMENT OF THE IDEA OF SOMETHING NEW:

Somewhere in this period of time ant hills began to appear at various locations. For the most part these were detected in the area of the property where the soil was kept relatively moist and was watered with some degree of frequency. Usually they made their appearance on the eastern edge of our property and it became quite easy to detect the hills moving in a westerly direction. This occurred whether or not a given hill had been treated with anything in any manner.

We contacted some of our neighbors to see if they were encountering a similar insect problem and they were!

During this period of time a host of treatment schemes were tried. Many of these were in the area of 'tried and true home styles'.   A typical listing would include grits, water from boiling potatoes, boiling water, vinegar (in widely varying strengths), hydrochloric acid (in widely varying strengths), Clorox Bleach Solutions, waste motor oils, gasoline, diesel fuels and a complete listing of all of the various chemical treatments being offered by Home Depot and various gardening supply houses.   These proved to be intermittently effective but quite expensive and difficult to apply uniformly.

It was also noted during this period of time that the method of application of a given treatment was of extreme importance to the results obtained. What eventually came out of this that it is absolutely essential that the 'egg generator' or 'queen' be knocked off as soon as possible. Knocking off the dear old girl just as quickly as possible and as effectively as possible then became my objective in life. The worst results, in these tests, were obtained by treating a hill with a teaspoon of powder or dusting an area. In general the best results were obtained by drenching the hill in question with the treatment involved.

It was also noted, during this period of time, that all of the native residents of my property had gone somewhere else. The fire ants even went after the rattle snakes! The quail disappeared. The frogs disappeared! The squirrel population dropped by about 50%. The rabbits were also less plentiful.

My wife then offered me my second incentive: "It was along the line of you are killing the fire ants but you are also killing everything else that is in the area of where you treat!"

Literal interpretation get 'in gear' and do something.

As a result of the above 'labors' it was noted that the most effective method of knocking down a fire ant hill was through the use of a oil of some type. It was also noted that undiluted oils were the most harmful to the surrounding areas. Dilute emulsions of various oils were then prepared to see which proved to be the most effective and the least harmful to the surrounding area.

DEFINITION OF THE PROBLEM:

As a result of the above work a program was set up to determine how to go about how to simply knocking off the queen of the colony. Here the question became something like she has got to be down there somewhere and she has got to be reasonably near her brood and accessible to the people who were responsible for keeping her fed and happy as it were.

A group of hills was then dig up. Various references indicated ants would move the queen just by someone walking above the mound; that disturbing the mound would also cause the ants to move the queen to a new location. Nothing was done that would allow verification of these statements. However, a method of estimating the volume of a fire ant hill was accomplished. This meant opening up the hill and flooding it with water and then recording the amount of water needed to flood the hill that was being evaluated.

The problem then was defined as follows: (1) Locate the ant hill, (2) Open the ant hill, and (3) Flood the ant hill as quickly and as effectively as possible.

LOOKING FOR A 'NATURAL' CURE

After having made, what might be termed progress, the Internet was searched for references. It was then found that most state institutions (agricultural, educational and others) had a multitude of references regarding fire ants a.k.a. Solenopsis Invicta + variations thereon. This took about six months because of the compounding of references. By compounding is mean the institutional habit of quoting a list of references at the end of each paper - many of which appeared in several more papers. Also involved in this is USDA

In one specific instance a local supplier of gardening materials was contacted and one package of every mixture they had in stock was purchased. These treatments were then applied in the local area. One way was to locate the homes that had small children. Mothers are acutely aware of fire ants and small

children.

Every system that was suggested either on the Internet or was suggested by someone involved in the sale of fire ant 'killers' - guaranteed or otherwise was purchased and tested at least once.

A lot of fire ants were killed by these experiments but the land area was never completely cleared of fire ants.

I am also sure that a lot of other things were killed that were around at the time of the treatment.

In one of the searches on the Web Site for US EPA came across Bulletin PR 2000-6 entitled Minimum Risk Insecticides. After studying this for some time the conclusion was reached that if one were to make an effective insecticidal system of a group of these products one would have a marketable product that would not require a US EPA Registration Number.

US EPA has a wonderful web site but it is extremely difficult to navigate and there are a lot of people in the organization and it is difficult to find one who is knowledgeable of the situation.

After about six months of experimenting a relatively stable emulsion of: (1) Water or Extract Liquor, (2) An aqueous solution of Sodium Lauryl Sulfate, (3) one of the oils mentioned in the above Bulletin and (4) a water solution of cane sugar molasses was produced This emulsion, when properly diluted and properly applied to a active fire ant hill managed to 'effectively kill' a large portion of the ants who came in contact with the emulsion. However, the initial joy soon turned to sadness as it was noted that ant activity could frequently be detected in the area of the hill after treatment Upon observing a set of hills for consecutive days it was noted that the ant activity on and around about 80% of the hills was gradually decreasing and ceased after about 7 to 10 days.

Flagging the hills so that they could be detected more easily was then instituted as a regular practice. Using this procedure a hill would be marked and treated and then revisited 7 - 10 days later. If ant activity was detected the hill would then be retreated. The second treatment invariably resulted in a complete kill.

It was interesting to note that new fire ants did not come back to an existing cavern. It was also interesting to note that 'satellite' hills did not form as they frequently did when one treated a hill with a powder insecticide.

The emulsion process was then repeated with other oils that are similar to those in US EPA Bulletin 2000-6 and the results obtained were similar in nature to those obtained when an emulsion was prepared from a material in Bulletin 2000-6.

THE DEVELOPMENT OF A METHODOLOGY OF TREATMENT:

Using the materials contained in PR 2000-6 a program was set up to treat fire ant hills with a single system but varying the method of application. After some period of time it became obvious that the method of application was almost as important as the treatment system. At the present time there are four systems of application that are functionally effective.

Suffice to say that a difference of a 70 % kill to a 90% or higher kill, using the same treatment system can be traced to the method of application used in the treatment.

THE RESULTS OF USING VARIOUS OILY MATERIALS:

After the above the conclusion was reached that a fire ant mound when treated with an aqueous emulsion of an oily substance in a contact effective manner could be killed within a short period of time. By this is meant the ants that were contacted with the oily emulsion would die shortly and - if the queen was contacted - the hill would die within a period of 7 to 10 days.

FIRST EXPERIENCE WITH PHYTOSIS:

Most of the above testing was accomplished in fields or in areas where decorative grasses were not established. When the program was moved into grassy areas it was noted that an immediate effect was the appearance of a 'sheen' on the blades of grass around the fire ant hill. When the 'lighter' oils were used this covering was reduced and the grasses remained essentially green. When 'heavier' oils were used this covering was intensified and the grasses would show symptoms of yellowing. This effect is phytosis.

The next question was to find a system that could be effectively used in grassy areas and which did not produce phytosis.

THE DEVELOPMENT OF BYE ANT!©.

One of the problems encountered was producing a mixture which contained a sufficient amount of oily substance that kill efficiency remained high. This meant that there was most likely a range of oil concentrations in the applied mixture that is effective. If one went too much above the upper limit the phytosis would become pronounced and if one went too far below the lower limit the phytosis effect would be minimal and the killing efficiency would be reduced.

This brought the problem back to the US EPA Bulletin. It was noted that one of the materials listed as being suitable was molasses. Mixtures of water and molasses have long been used as a soil adjuvant by commercial farmers. No type was specified so experimentation was done with emulsions that contained various molasses and/or sugars. As the concentration of molasses in the applied fluid went up the amount of phytosis encountered went down.

EXPERIENCES WITH BYE ANT!

The author treated a series of potted trees in containers of 15 to 25 gallons. All of the containers were severely infested with fire ants and were not a sellable product. These trees were not for export as they had been grown completely in the nursery in question. The trees were each treated with 2 - 3 gallons of dilute mixture that was mixed in a plastic bucket and simply poured over the surface of the container so that the container was more or less flooded at the top. Inspection of the trees at intervals of 1, 2, 3, & 4 weeks showed that there were no fire ants present so the trees were put up for sale and sold. Three or four trees were treated again at the end of this period of time.

In most of Parker County the fire ant hills are relatively small. Treatment volumes of one-half, one, two and four gallons have been used. A rough guess is based upon the size of the mound above the colony with a hill of 3 - 4 inches diameter requiring about one half gallon of diluted material. Depending upon the care used in the application of the material the mound kill rate is on the order or 80 to 90 %. What is seen is a change in the life around the hill over a period of 4 - 10 days with a sudden drop in population and then a gradual disappearance of any activity over the time noted. To date there has been no indication that satellite mounds are formed as a result of the treatment. To date there is no indication that phytosis of the grasses around the treatment area is a problem.

The author has used very dilute mixtures of the material in the family greenhouse for the control of spider mites and other infestations (such as mealy bugs) that are common to a small greenhouse (14 feet x 16 feet). These high dilutions have been used with what is considered to be good success.

SOME OBSERVATIONS ABOUT TREATED AREAS:

In the specific case of our 2.5 acres it has been noted that most of the ground life has returned to a more or less normal status. This year the frogs (or toads) returned to some extent. Since the year has not been one that is over wet it is assumed that the presence of these creatures was occasioned by the disappearance (or control if you prefer) of the fire ant population.

PRESENT STATUS

U. S. Patent Number 6,699,489 dated March 2, 2004 has been issued to R. E. Driscoll, Sr.

A semi-commercial unit has been constructed in Weatherford, TX and the production of BYE ANT! has begun. Case quantities of the product are available and priced FOB Weatherford. Shipment can be via U. S. Postal Services (only in small amounts) UPS and FEDEX.

Mr. Ettinger has been licensed to produce and sell the products in the States of Florida, Georgia and South Carolina.

Mr. Mayes has been licensed to produce and sell the products in Louisiana and Mississippi.

Mr. Driscoll is working in the State of Texas.

Authors:

R. E. Driscoll, Sr.:

Mr. Driscoll graduated from the Missouri School of Mines and Metallurgy, Rolla, MO in May 1949. He has been associated with the petrochemical industry since that time and for about 35 years was specifically associated with the carbon black industry. He is the holder of 16 patents during that period of time. He retired in 1988 and acted as a carbon black consultant to various firms in China, Indonesia and India. He has also acted as a consultant to the synthetic rubber industry.

Hugh M. Ettinger:

Mr. Ettinger graduated from the Amos Tuck School of Business Administration, Dartmouth College, Hanover, N.H. in 1948. He worked as a securities analyst for a quarter century, after which he spent another quarter century with Bedminster Bioconversion Corporation, a company that uses compartmented rotary kilns to convert unsegregated municipal solid and liquid wastes to compost. There are a dozen plants around the world that employ this technology, the largest of which processes 600 tons a day of municipal solid waste with dewatered biosolids. Mr. Ettinger is now retired and devotes his time to consulting work and the promotion of the Bye Ant! technology.

M. Dale Mayes: Mr. Mayes graduated from Louisiana State University.

Detection of fire ant mounds in airborne digital images: Hierarchical learning for automatic feature extraction.[1]

James T. Vogt
USDA, ARS Biological Control of Pests Research Unit
PO Box 67
Stoneville, MS 38776

Abstract. Quantifying imported fire ant mounds over large areas is expensive and time-consuming. New methods of detecting and quantifying mounds will be useful for researchers engaged in regional management projects, assessment of biological control agents following release, and examination of landscape effects on fire ant populations. Regulatory personnel tracking new introductions and spread of fire ants will also benefit. Airborne digital imagery can be used to detect >70% of imported fire ant mounds in pasture areas, but photointerpretation of imagery is time-consuming. Use of commercially available software for supervised classification of images resulted in average detection of >60% of mounds in airborne imagery, with no commission errors.

Introduction

Remote sensing technology may yield tremendous benefits to researchers engaged in projects that require quantification of imported fire ant *(Solenopsis invicta* Buren, *S. richteri* Forel, and their hybrid) colonies over large areas. Early, airborne film-based work by Green et al. (1977) over Texas coastal prairie resulted in varying detection rates with different film types. Up to 79.4% of colonies were detected using photointerpretation of color infrared film. Fire ant mounds created a "dark spot-red halo" signature in images. This resulted from freshly excavated mound soil surrounded by the typically lush vegetation growing at the periphery of the mound; healthy vegetation typically exhibits high reflectance in the near infrared portion of the spectrum, while soil exhibits low reflectance (unless extremely dry) (Jensen 2000).

A test of airborne multispectral digital imagery for mound detection over Mississippi pasture resulted in detection rates (using photointerpretation) of about 70%, and demonstrated that the dark spot-red halo signature was evident in 0.25 to 0.1 m spatial resolution, false color infrared imagery (Vogt 2004). Mound characteristics (size, vegetation cover, and activity) all influenced detection rates during some part of the year, and mound signature changed with season. The purpose of this study was to determine the feasibility of using commercially available software (Feature Analyst™, Visual Learning Systems, Inc., Missoula, MT) for supervised classification of mounds. Feature analyst combines machine learning algorithms such as nearest neighbor, neural networks, decision trees, and genetic ensemble feature selection (Pat. Pending) to classify user-specified features. It is capable of iteratively improving classification through hierarchical learning methods, and uses foveal vision (Pat. Pending) (after the foveal centralis, or the area of the retina associated with the most acute vision) to examine spatial context of features (Visual Learning Systems, Inc. 2002).

---

[1] Mention of specific products or trade names does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Materials and methods

Data used for this study were acquired in May 2002 (Vogt 2004). Briefly, imagery was obtained by Geodata Airborne Mapping and Measurement, Inc. (Macon, Mississippi, USA) at an altitude of 305 m (0.1 m resolution) over a portion of a pasture in Clay County, Mississippi. A GeoScanner camera system (GeoVantage™, Inc., Swampscott, MA), mounted on a Cessna 172 aircraft, consisted of four discrete monochrome digital cameras with 10 nm band-pass filters (450, 550, 650, and 850 nm). A GPS antenna and 12-channel receiver were installed on the plane and integrated with the data collection system, and an Inertial Measurement Unit (IMU) provided acceleration and rotation rates for the camera axes. Individual, georeferenced frames were used for the purposes of this study.

Frames containing previously georeferenced plots (N = 3) were used for analysis. Each plot was about 0.4 ha; each fire ant mound within a plot was georeferenced within 2 weeks of data acquisition. Mound characteristics (activity, size, vegetation cover) were also recorded but not used in the following analysis.

A series of transformations were applied to the data to increase contrast and enhance bare mound soil and peripheral vegetation in the images. Based on visual appearance of the images and examination of reflectance values for pixels representing mound soil and peripheral vegetation, I built a false color composite consisting of a simple vegetation index (Birth and McVey 1968) and band ratios (Table 1). Transformations were done using ENVI® software (The Environment for Visualizing Images, Research Systems, Inc., Boulder, CO) and transformed frames were imported into ArcMap 8.2 (ESRI, Inc., Redlands, CA) for classification.

Table 1. Image transformations for enhancing fire ant mound features.

Enhanced Feature	Transformation[1]	Display Color
Mound Soil	ρBlue / ρGreen	Red
Peripheral Vegetation	ρNIR / ρRed	Green
Other	ρRed / ρGreen	Blue

[1]ρ = reflectance value (0-255), NIR = near infrared (840-860 nm).

Plots contained 18-24 georeferenced mounds. The first step in analysis with Feature Analyst was digitizing a training set (N = 6-8) of mounds. Next, a foveal input representation was chosen for learning (Figure 1). The pattern chosen was 27 x 27 pixels, with a 9 x 9 pixel arrangement in the center. The central pattern was chosen because it approximated the size of large mounds in the images. The program examined each pixel in an image in the context of the surrounding pattern, as specified above, and classified the image. After initial classification, I tallied correct classifications, omission errors, and commission errors, based on georeferenced mound locations. Hierarchical learning features were then applied to the data. Several (N = 6-8) classified features were chosen in each image for each of three categories: correctly classified, commission errors, and omission errors. After a second iteration, I again tallied correct classifications, omission errors, and commission errors.

Results

Results are summarized in Table 2. With a single iteration, Feature Analyst classified a large percentage of mounds, but commission errors resulted in the possibility of significantly overestimating the number of actual mounds present. Many commission errors were due to small patches of vigorous vegetation in the absence of fire ant mounds. After designating correctly classified mounds, commission errors, and omission errors and running a second iteration, mound classification was similar, but potential overestimation due to commission errors was greatly reduced.
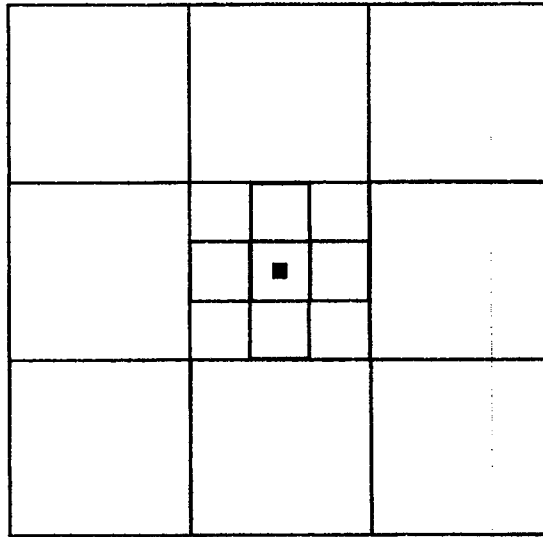
Figure 1. Foveal input representation used to classify fire ant mounds in airborne images with Feature Analyst.

Table 2. Classification of mounds in airborne imagery of Mississippi pasture using Feature Analyst.

Iteration	Percent Mounds Classified	Potential Percent Overestimation
1	62.3 ± 12.9	29.2 ± 4.2
2	66.8 ± 12.0	0

Discussion

Feature analyst detected nearly as many fire ant mounds as a human photointerpreter in a previous study (Vogt 2004). Background is likely to influence classification of small, temporally variable (Vogt et al. 2004) targets such as fire ant mounds, so it is not clear whether Feature Analyst will be useful for classifying large images with heterogeneous landscape. Additional work is planned using ENVI to further automate detection by classifying large images according

to landscape, digitizing training sets within each landscape, and automatically examining individual frames within landscape classes.

References Cited

Birth, G. S., and G. McVey. 1968. Measuring the color of growing turf with a reflectance spectrophotometer. Agronomy Journal 60: 640-643.

Green, L. R., J. K. Olson, W. G. Hart, and M. R. Davis. 1977. Aerial photographic detection of imported fire ant mounds. Photogrammetric Engineering and Remote Sensing 43: 1051-1057.

Jensen, J. R. 2000. Remote sensing of the environment: An Earth resource perspective. Prentice-Hall, Inc., Upper Saddle River, NJ. 544 pp.

Visual Learning Systems, Inc. 2002. The Feature Analyst™ Extension for ArcView® and ArcGis™. A Visual Learning Systems, Inc. White Paper (http://www.vls-inc.com/downloads/whitePapers/FA_whtpapr.pdf) (last accessed 03-04-2004).

Vogt, J. T. 2004. Quantifying imported fire ant (Hymenoptera: Formicidae) mounds with airborne digital imagery. Environmental Entomology (in press).

Vogt, J. T., D. A. Streett, and D. A. Boykin. 2004. Seasonal characteristics of black imported fire ant (Hymenoptera: Formicidae) mounds in northern Mississippi pastures. Sociobiology 43: 1-10 (in press).

161 **Host and Parasite Interactions between the Red Imported Fire Ant and a Microsporidian Parasite.**
M. Walker Hale and S.B. Vinson. Texas A&M University

162 **PCR-based Analysis of Spores Isolated from Smears by Laser Pressure Catapult Microdissection Techniques Confirms Genetic Identity of Spore Morphotypes of *Thelohania solenopsae.***
Yuliya Y. Sokolova, Lacey R. McNally, and James R. Fuxa.
Louisiana State University, LSU AgCenter

163 **Evaluation of Broadcast Applications of Cyfluthrin and Beta-cyfluthrin Against Red Imported Fire Ants (Solenopsis invicta).**
Kelly Loftin, John Hopkins, Doug Petty, and Donna Shanklin. UA-CES

164 **Evaluation of Selected Insecticides as Individual Mound Treatments Against Red Imported Fire Ants.** Kelly Loftin, John Hopkins, Allan Beuerman. UA-CES

165 **Kid₂Ants®: A New Curriculum about Fire Ants for Kids.** Nathan Riggs, Elizabeth Brown, Paul Nester. Texas Cooperative Extension

167 **Interstate Collaborative Efforts to Develop Educational Programs for Fire Ant Management in Cattle Production Systems.**
Flanders, K.L. and B.M. Drees.

169 **RIFA, *Solenopsis invicta* Management in a South Louisiana Citrus Orchard.**
Dale Pollet, Patricia Beckley, and Boris Castro, Louisiana State University, LSU AgCenter

171 **Initiation of Flight Muscle Histolysis in the Red Imported Fire Ant (*Solenopsis invicta*).** T. Azizi and S.B. Vinson. Texas A&M University

172 **Gene Overexpression Associated with Workers of the Red Imported Fire Ant, *Solenopsis invicta* Buren.** Nannan Liu and Lee Zhang. Auburn University

174 **Red Imported Fire Ant: Prevention "Plans" in the Pacific**
Carol Russell, USDA/APHIS/PPQ

Abstract for poster for RIPA Conference, Baton Rouge, Louisiana Mar 21 -23, 2004

Title: Survey of the Ants of Alabama

Jason A. Forster and Michael **L.** Williams. Auburn University, Entomology and Plant Pathology, 301 Funchess Hall, Auburn, AL

Preliminary findings of ant species occurring in the state of Alabama.

The only published state wide survey of ants in Alabama was completed in 1947 by L. C. Murphree as part of his Master's thesis. Murphree's study, which was limited to species found as urban pests, listed 47 species in 19 genera . Our ongoing project explores in more detail what species are present in both urban and rural environments. The survey involves collecting ants on a county by county basis keeping physiographic regions in mind. Collection methods include: hand collecting, baiting with various food types, use of pit fall traps, soil sampling using Berlese funnel extraction technique, and night collecting using UV lights to attract males.

Biodiversity and Distribution of the Ants of Alabama

Jason A. Forster and Michael L. Williams

Department of Entomology and Plant Pathology

301 Funchess Hall, Auburn University, AL 36849

Abstract

The purpose of this project is to explore and expand upon the knowledge of the ant fauna of Alabama. The last survey was completed in 1947 and at least 2 species have been displaced. To date, 55 species have been identified. Specimens have been collected from 56 of 67 counties in Alabama.

Introduction

The first and only statewide survey of the ants of Alabama was completed in 1947 by L.C. Murphree as part of a Master of Science thesis project. Murphree most likely collected his specimens in the 1930's while performing part of a USDA study on the range of the Argentine Ant, *Linepithema humile* (Mayr). This survey focused mainly on urban areas and the author collected 47 recognized species in Alabama (subsequent taxonomic changes and synonymy have reduced this number to 41 species). Murphree estimated that the actual number of species in the state should approach 125 since neighboring states reported approximately that number.

According to Murphree's data, the Imported Fire Ant (probably the Black Imported Fire Ant, *Solenopsis richteri* Forel), was only found in 16 localities in southern Alabama (confined to Baldwin and Mobile county). Two other Fire Ant species that are thought to be native to North America were reported: *Solenopsis xyloni* McCook with a statewide distribution and *Solenopsis geminata* (Fab.) in southern Alabama.

In 1976, Glancey, et al. performed a bait transect study of the ants of Mobile county and found a total of 16 species. At this point the Red Imported Fire Ant dominated the ant fauna of Alabama and no signs of the once abundant *S. xyloni* or *S. geminata* were found.

Materials and Methods

Specimens were collected by various methods including hand collecting, Berlese funnels, baits, light traps and pit fall traps. Museum and literature records are also being examined. After the collected ants are pointed and identified they are deposited in the Auburn University Entomological Museum Collection (AUEM). All species identifications will be verified by checking against representatives in other collections.

Discussion

Of the 55 species identified 22 were not reported in the 1947 survey. Table 1 presents a list of the ant species collected and identified to date. This is an ongoing project, and some specimens collected during the summer of 2003 are still being processed.

Table 1: List of all species identified from the 2003 field season with species collected by L. C. Murphree in 1947 marked by *.

Literature Cited

Glancey, B. M., C. P. Wicht, C. H. Craig, and J. A. McNeil. 1976. Ants of Mobile county, AL as monitored by bait transects. J. Georgia Ent. Soc. 11(3): 191-197.

Murphree, L. C. 1947. Alabama ants, their distribution, identification and proposed control. Mississippi State College. 1947.

DISTRIBUTION OF IMPORTED FIRE ANT POPULATIONS IN ALABAMA

L.C. 'Fudd' Graham[1], V.E. Bertagnolli[2] and R.K.Vander Meer[3]
Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama [1,2]
USDA-ARS, CMAVE, Gainesville, Florida [3]

Introduction

When fire ants were introduced into Alabama in the early 1900's, almost all of their natural enemies were left behind in South America (Jouvenaz 1990). As a result, fire ant densities are much higher in Alabama than they are in South America (Porter et al. 1997). Two species of imported fire ant occur in Alabama. The red imported fire ant, *Solenopsis invicta,* is located in the southern portion of the state and the black imported fire ant, *Solenopsis richteri* , is located in northwest Alabama.

Although the black imported fire ant was introduced into the United States before the red imported fire ant, its current range is thought to be northeastern Mississippi and northwestern Alabama. Vander Meer et. al. (1985) first detected a hybrid between the two species in Mississippi. The hybrid is thought to populate the northern tier of Alabama, Mississippi and Georgia .

One group of natural enemies that have shown some promise in the battle against fire ants are phorid flies in the genus *Pseudacteon* (Porter 2000). Currently, nine populations of phorids have been successfully established in Alabama. *Pseudacteon tricuspis* is established at five sites in *S. invicta* populations and shows a strong preference for *S. invicta. Pseudacteon curvatus* is established at five sites in hybrid fire ant populations and shows a strong preference for *S. richteri* .

We are planning several new releases of these same species in 2004. We hope to obtain *Pseudacteon litoralis* and the biotype of P. *curvatus* that is host specific to *S. invicta* when these two species are available for release. The purpose of this study is to determine the location of each imported fire ant species in Alabama. This will allow us to release phorid flies on their preferred host.

Methods and Materials

A grid was superimposed on a map of Alabama. The grid is an extension of the one used by Diffie et al. (2002) in Georgia. The squares are approximately 27 km x 27 km. Worker ants were collected from three mounds at or near the intersection on the grid. These sites were surveyed during 2003-2004.

Ants were collected from each site by inserting a 30 x 80 mm plastic tube into a mound and capping it once at least 25 ants fell into the tube. Ants were chilled and approximately 25 were removed from the sample tube. These were placed into seven ml glass scintillation vials and covered with hexane. After 24 hours, the hexane was removed, added to a clean seven ml scintillation vial, and allowed to evaporate. These vials containing cuticular hydrocarbon residues from the ants were shipped to CMAVE in Gainesville, FL for species determination (Vander Meer et al. 1985).

References

Diffie. S., R. K. Vander Meer, and W. Gardner. Range of the hybrid imported fire ant in Georgia, pp 164-167. In Proceedings of the 2002 Imported Fire Ant Conference. Athens, GA.

Jouvenaz, D. P. 1990. Approaches to biological control of fire ants in the United States. *In* "Applied Myrmecology: World   Perspective" (R. K. Vander Meer, K. Jaffe, and A. Cedeno, Eds.), pp. 620-627. Westview Press, Boulder CO.

Porter, S. D., Williams, D. F., Patterson, R. S., and Fowler, H. G. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): Escape from natural enemies? Environ. Entomol. 26 :373-84.

Porter, S. D., 2000. Host specificity and risk assessment of releasing the decapitating fly *Pseudacteon curvatus* as a classical   biocontrol agent for imported fire ants. Biol. Control 19: 35-47.

Vander Meer, R. K., C. S. Lofgren, and F. M. Alvarez. 1985. Biochemical evidence for hybridization in fire ants. Florida Entomol.   68:501-506.
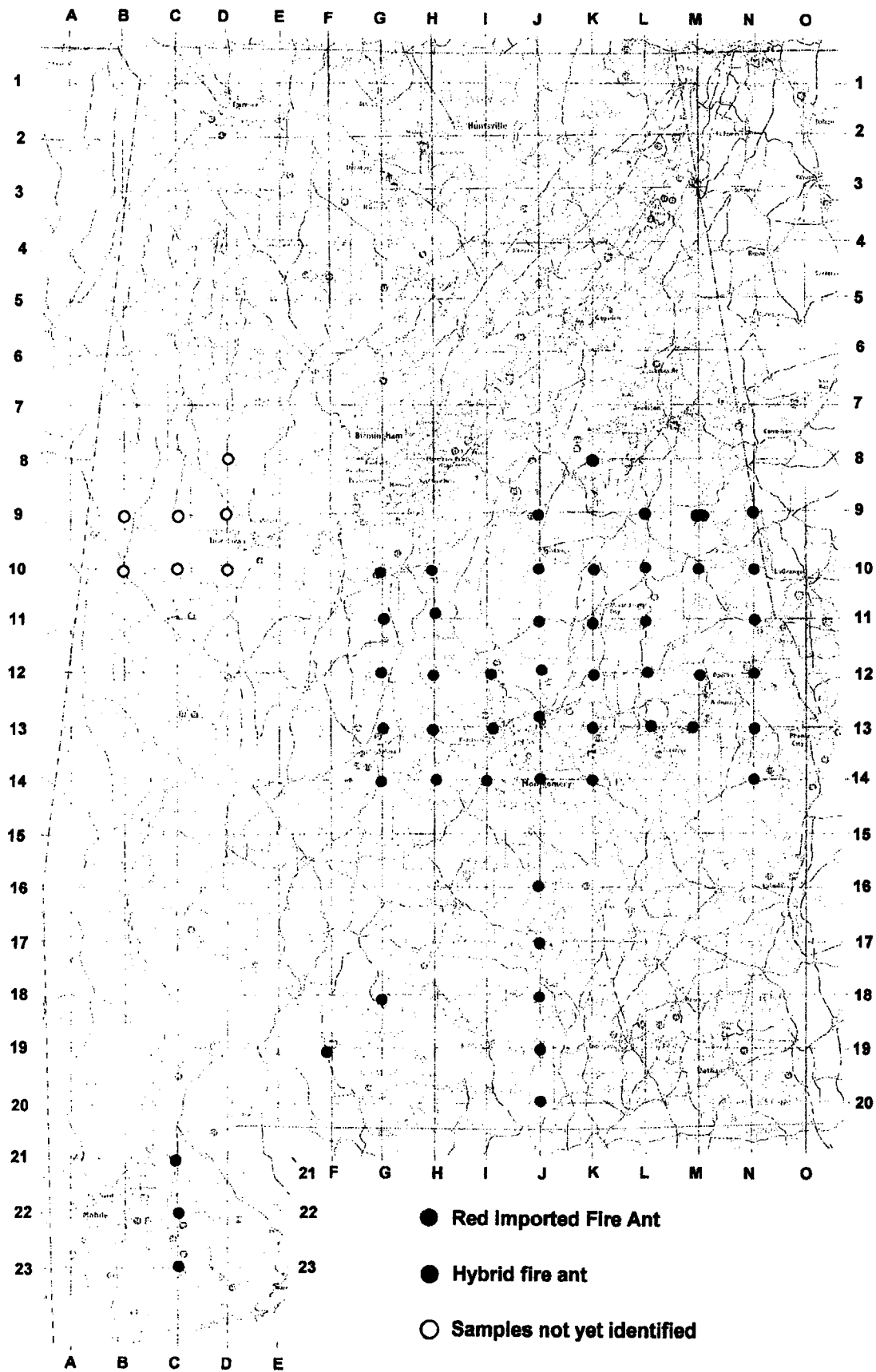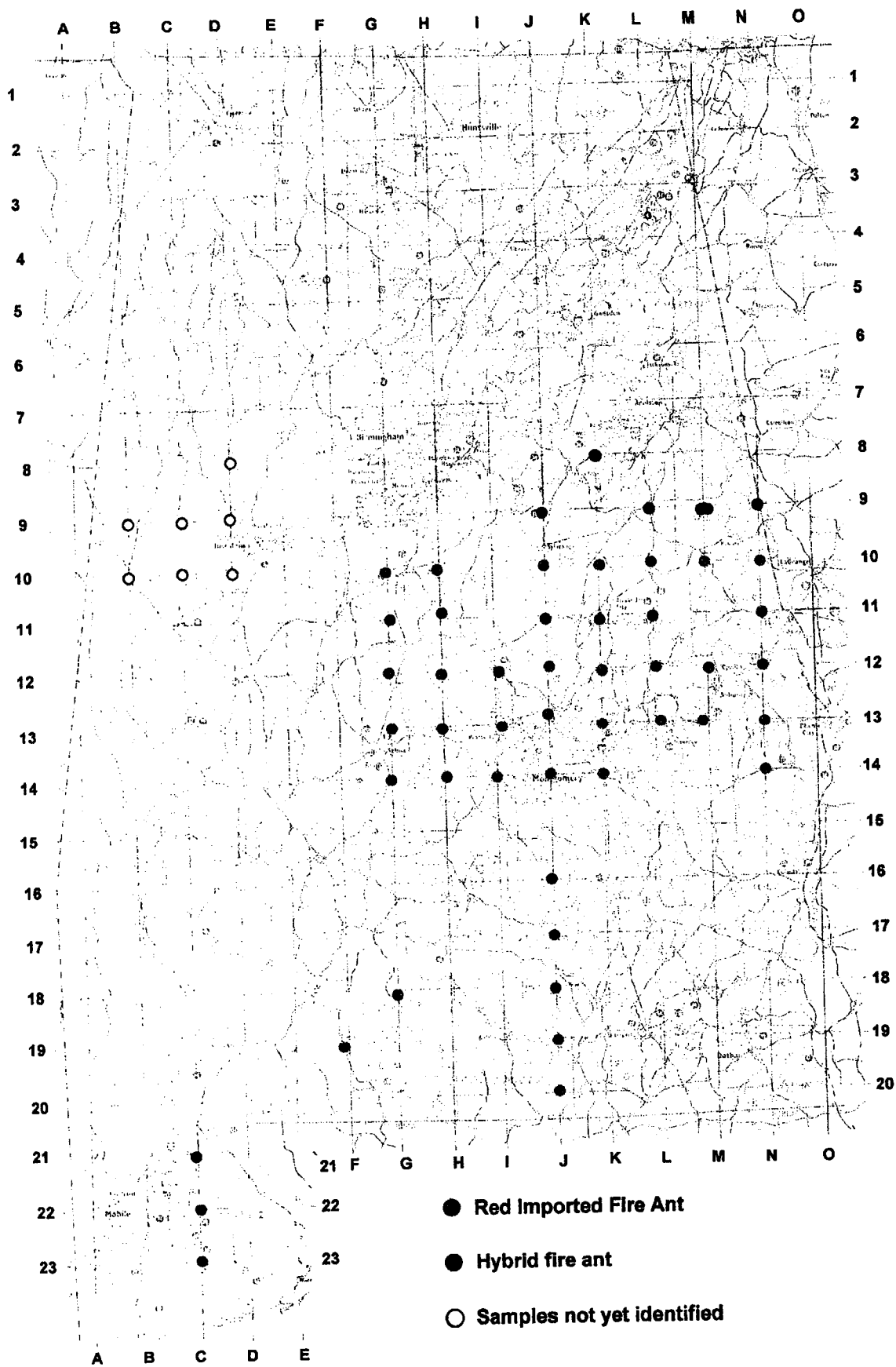
Red Imported Fire Ant

Hybrid fire ant

Samples not yet identified

●	Red Imported Fire Ant			
●	Hybrid fire ant			
○	Samples not yet identified			

Dispersal of the decapitating fly, *Pseudacteon tricuspis* Borgmeier, a biological control agent of the red imported fire ant *Solenopsis invicta* Buren in southeast Louisiana

D.C. Henne[1], S.J. Johnson[1] and S.D. Porter[2]

[1] Department of Entomology, 402 Life Sciences Building, Louisiana State University Agricultural Center, Baton Rouge, LA, 70803, [2] USDA-ARS Center for Medical, Agricultural and Veterinary Entomology. Imported Fire Ant and Household Insects Research Unit, 1600 SW 23rd Drive, Gainesville, FL 32608

Abstract

Establishment of *Pseudacteon tricuspis* has been confirmed at the following release locations in SE Louisiana: (1) −13 km north of Covington (St. Tammany Parish), (2) −8 km east of Norwood (East Feliciana Parish). During September and October, dispersal of P. *tricuspis* was evaluated in four cardinal directions from the release point (N, S, E, and W), at increasing distances until no flies were observed. At each evaluation site, 8-12 S. *invicta* mounds were disturbed and monitored for 30 minutes. *Pseudacteon tricuspis* have dispersed almost 14.5 km from the Covington release site since fill 1999 and almost 10km from the Nonvood release site since spring 2000. Average number of flies observed per S. *invicta* mounds in fall 2003 were 0.69 flies/mound at the Covington release site and 1.04 flieslmound at the Nonvood release site. The rate of dispersal at the Covington release site has not yet reached an asymptotic rate, implying that the rate of dispersal is increasing.

Dispersal of the decapitating fly, *Pseudacteon tricuspis* Borgmeier, a biological control agent of the red imported fire ant *Solenopsis invicta* Buren in southeast Louisiana

D.C. Henne[1], S.J. Johnson[1] and S.D. Porter[2]

INTRODUCTION

Establishment of *Pseudacteon tricuspis* has been confirmed at the following release locations in SE Louisiana: (1) ~13 km north of Covington (St. Tammany Parish), 2,165 flies released 6-13 September, 1999; (2) ~8 km east of Norwood (East Feliciana Parish) ~4,700 flies released April 27-May 8, 2000. Additional releases were made in St. Helena Parish (3) in 2000, Vermillion Parish (4) in 2001, Richland Parish (5) in 2001 and Rapides Parish (6) in 2002. Establishment has not been confirmed at release sites 3-6.
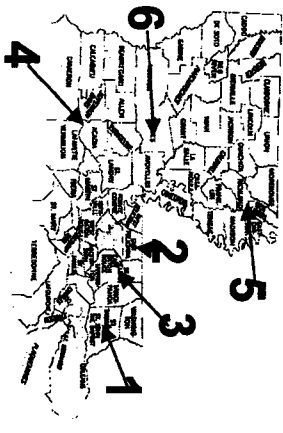
MATERIALS AND METHODS

At each dispersal evaluation site, 8-12 *S. invicta* mounds are disturbed. Styrofoam plate shades (below) help to maintain ant activity on the surface. Mounds are monitored for 30 minutes and the number of *P. tricuspis* counted. Dispersal was evaluated in four cardinal directions from the release point (N, S, E, and W), at increasing distances until no flies were observed.

RESULTS

Table 1. Approximate *P. tricuspis* dispersal distances (km), Norwood and Covington, LA release sites; September-October 2003.

Release Site	Direction and approximate dispersal distance (km)			
	North	South	East	West
Covington	12.53	11.77	14.38	8.66
Norwood	9.77	1.63	3.46	1.63

Figure 1: *Pseudacteon tricuspis* dispersal map–Covington, LA 2000-2003.

Dispersal distances of *P. tricuspis* at Covington and Norwood, LA release sites (Table 1). Note the directional heterogeneity of dispersal at these sites, particularly to the north and east of the release sites. The resulting distribution is ellipsoid. Thus, calculations of dispersal area follow formula for an ellipse: L/2 x W/2 x π, where L=North-South diameter and W=East-West diameter. The map on which dispersal distances have been overlaid does not explain this heterogeneity on the basis of spatial characteristics. However, several hurricanes have passed through these areas since the initial release, which might explain a possible source for this heterogeneity. This contrasts Morrison et al. (1999) and Porter et al. (2004), who reported that dispersal of *Pseudacteon* spp. were not related to wind patterns.

Figure 2: A regression of square root of area containing *P. tricuspis* around Covington, LA release area, 2000-2003.
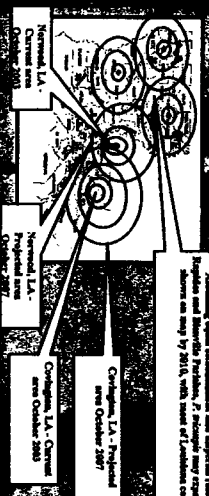
Figure 3: Projected dispersal of *P. tricuspis* from the Covington and Norwood LA release sites, 2004-2007. Projected dispersal of other release sites in central and north-central Louisiana are shown as well. Projections based on dispersal rates at Covington. Asymptotic rate of spread is unknown at this time.

Table 2. Number of *S. invicta* mounds disturbed and numbers of *P. tricuspis* observed. Counts shown are from study areas in dispersal zones only, Covington and Norwood, September-October 2003.

Location	Total # mounds disturbed	Total # flies at mounds	Average # flies/mound
Covington	100	69	0.69
Norwood	113	117	1.04

REFERENCES CITED
Morrison, L.W., E.A. Kawazoe, R. Guerra and L.E. Gilbert. 1999. Phenology and dispersal in Pseudacteon flies (Diptera: Phoridae), parasitoids of Solenopsis fire ants (Hymenoptera: Formicidae). Ann. Ent. Soc. Am. 92(2): 198-207.
Porter, S.D., L.A. Nogueira de Sá and L.W. Morrison. 2004. Establishment and dispersal of the fire ant decapitating fly Pseudacteon tricuspis in North Florida. Biol. Cont. 29(2): 179-188.

1 Department of Entomology, 402 Life Sciences Building, Louisiana State University Agricultural Center, Baton Rouge, LA, 70803

2 USDA-ARS Center for Medical, Agricultural and Veterinary Entomology. Imported Fire Ant and Household Insects Research Unit, 1600 SW 23rd Drive, Gainesville, FL 32608

LSU AgCenter Research & Extension

Pesticide Surface Treatments Applied to Fire Ant Mounds During April as Potential Nursery Quarantine Treatments – 2003 Tennessee Trials

K. Vail[1], J. Oliver[2], C. Kimbro[3], D. Bailey[1], J. Morton[1], N. Youssef[2], P. Parkman[1], S. James[4] and A.- M. Callcott[4]

[1] The University of Tennessee, Department of Entomology and Plant Pathology, 205 PSB, Knoxville, TN 37996

[2] Tennessee State University, IAER. Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110.

[a] The University of Tennessee Agricultural Extension Service, Grundy County Extension, HWY 56 & Phipps Street, Coalmont, TN 37313-0338

[4] USDA-APHIS-PPQ, Soil Inhabiting Pests Laboratory, 3505 25th Avenue, Gulfport, MS 39501.

Abstract

Effective, cool-weather insecticide treatments are needed for nursery producers to eliminate imported fire ants from nursery stock before shipping and to satisfy quarantine requirements. Ideally, treatments must have contact activity and be able to move into the soil profile. The pyrethroids, Talstar™ GC Flowable, Scimitar™ GC, and Deltagard™ GC 5SC, applied in April at broadcast rates, were evaluated. Talstar™ required 6 weeks to completely eliminate IFA; however, no ants were found during the remainder of the monitoring period (12 additional weeks) in Talstar™-treated mounds. Deltaguard™ and Scimitar™ eliminated IFA activity by 3 and 4 weeks, respectively, but ants were found in some mounds periodically through the end of the monitoring period. Mounds receiving the water control survived better than those receiving the no-water control.

Pesticide Surface Treatments Applied to Fire Ant Mounds During April as Potential Nursery Quarantine Treatments – 2003 Tennessee Trials

K. Vail[1], J. Oliver[2], C. Kimbro[3], D. Bailey[1], J. Morton[1], N. Youssef[2], P. Parkman[1], S. James[4] and A.- M. Callcott[4]

[1] The University of Tennessee, Department of Entomology and Plant Pathology, 205 PSB, Knoxville, TN 37996
[2] Tennessee State University, IAER. Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110.
[3] The University of Tennessee Agricultural Extension Service, Grundy County Extension, HWY 56 & Phipps Street, Coalmont, TN 37313-0338
[4] USDA-APHIS-PPQ, Soil Inhabiting Pests Laboratory, 3505 25th Avenue, Gulfport, MS 39501.

Abstract

Effective, cool-weather insecticide treatments are needed for nursery producers to eliminate imported fire ants from nursery stock before shipping and to satisfy quarantine requirements. Ideally, treatments must have contact activity and be able to move into the soil profile. The pyrethroids, Talstar™ GC Flowable, Scimitar™ GC, and Deltagard™ GC 5SC, applied in April at broadcast rates, were evaluated. Talstar™ required 6 weeks to completely eliminate IFA; however, no ants were found during the remainder of the monitoring period (12 additional weeks) in Talstar™-treated mounds. Deltaguard™ and Scimitar™ eliminated IFA activity by 3 and 4 weeks, respectively, but ants were found in some mounds periodically through the end of the monitoring period. Mounds receiving the water control survived better than those receiving the no-water control

Introduction

Unlike most of the southeast, which is infested by the red imported fire ant (*Solenopsis invicta* Buren), Tennessee is infested primarily with the black imported fire ant (*Solenopsis richteri* Forel) and the hybrid of the black and the red species. Imported fire ants are now present in southern counties of Tennessee and occupy ca. 3.3 million hectares in the state. Fire ants are easily moved with containerized or field nursery stock, as well as in hay and pine straw, or on equipment that has soil attached. Thirty-four counties in the state are quarantined (see figure below). Consequently, any area with fire ants will be required to meet quarantine requirements before shipping nursery stock.

Tennessee fire ant quarantine areas. Shaded counties are major nursery producers.

Nursery producers in Tennessee ship nursery stock primarily during the fall – spring months when trees are dormant (October to April). These cooler times of the year are the most difficult time to attempt fire ant control with insecticides, because products are less likely to contact the ants. This is due to: 1) less foraging activity, 2) greater colony depth, and 3) slower ant metabolism. Bait products require active foraging by fire ants. Because fire ants forage optimally at temperatures between 16 and 33°C, baits typically are not effective during these cooler months. Insecticides that are likely to work in these cooler months are those with contact activity and the potential to move into the soil profile.

Fire ant mound around tree base.

For quarantine treatment, any product that kills the entire colony or induces the colony to move from the nursery row has potential. To be adopted by nursery producers, products also must be easy to use and not require large volumes of water for application. Pyrethroids, applied at broadcast rates are for other insects, meet these needs. Broadcast rates are typically lower and require less water for application than pesticides applied as individual mound treatments for IFA. Several products, which had been tested during the winters of 2000/2001 or 2002/2003, were further evaluated to determine their potential for quarantine treatment during the April shipping season. If these rates are effective, management costs to nursery growers, as well as potential pesticide exposure to workers, handlers and the environment, could be reduced.

The objective of the study was to determine the efficacy of pyrethroid insecticides applied at broadcast rates against IFA. This study was done in conjunction with trials begun earlier in the year at other TN sites and in Mississippi (and reported on at this Conference). Later in 2003, the Fire Ant Research and Education Team (FARET), a collaborative effort of the The University of Tennessee Institute of Agriculture, the Tennessee State University Institute for Agricultural and Environmental Research, and USDA-APHIS was formed. FARET's primary mission is to identify effective treatments or management alternatives that will eliminate IFA from nursery stock in order to meet quarantine requirements.

Materials and Methods

Three pyrethroid insecticides, applied as drenches, were evaluated for IFA control at a field nursery site in Grundy County, TN. (See table below for product and application information.) Applications were made on April 2, 2003.

Treatment	Active Ingredient	Rate of Application	Number of Mounds
Talstar™ GC Flowable	bifenthrin	3.7 g a.i./ha	5
Scimitar™ GC	lambda cyhalothrin	16.2 g a.i./ha	5
Deltagard™ GC-5SC	deltamethrin	2.8 g a.i./ha	5
Control		No application	4
Control-Water	water	Water only	4

Treatments and number of mounds used per treatment.

Immediately before application, active IFA mounds were identified by disturbing them with a trowel. Mounds were rated based on worker activity and presence of brood (Harlan et al. 1981). Mounds were flagged and marked with a stake (not in mound). Mound location was recorded using a GeoExplorer™ II GPS unit. Four or five mounds were used per treatment and controls.


A trowel was used to gently lift soil in the mound to determine activity and give a rating from 1-6 without brood or 6-16 with brood.

To delineate the treatment area (0.667 m²) and to determine future mound movement, four or five lines were painted along the circular perimeter of a 91 cm-diameter hula-hoop. The treatments were applied in 7.6 L of water, with at least 3.8 L applied directly to the mound.

Marking treatment perimeters using spray paint and a hula-hoop.
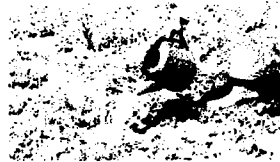
Applying a drench treatment.

Treated areas were checked weekly for the presence or absence of active mounds. Mounds were also rated. If needed, a meter stick was used to quickly determine whether an active mound was within the limits of the test circle. Plot monitoring continued for 18 weeks post-treatment.

Results

Based on ant activity and mound ratings, Talstar™ GC Flowable had completely eliminated IFA by 6 weeks post-treatment (see figures below). No ants were found in Talstar™-treated mounds for the remainder of the study (12 additional weeks). Deltaguard™ GC 5SC and Scimitar™ GC eliminated IFA activity by 3 and 4 weeks respectively, but ants were found in some mounds periodically through the end of the monitoring period. Mounds receiving the water control survived better than those receiving the no-water control.
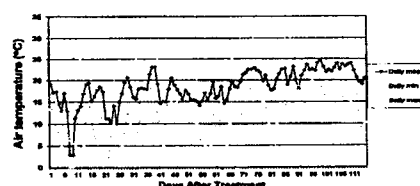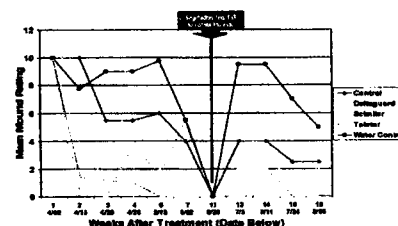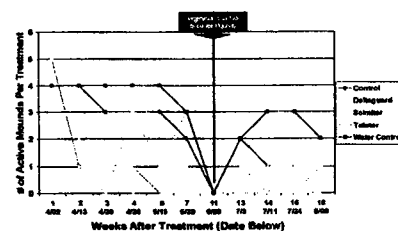
Discussion

Treatments were made late in the traditional nursery shipping season and at temperatures warmer than usually experienced during most of the shipping season (see weather data above). This may have affected results, reducing effectiveness of treatments. Of the treatments, Talstar™ GC Flowable appeared most promising, indicating that it might serve as an effective individual nursery plant treatment. In a companion study begun earlier (February 5) in neighboring counties, Deltaguard™ GC 5SC and Scimitar™ GC provided better control than that observed in this trial. More field trials of similar products are scheduled for winter 2004.

Reference cited

Harlan, D.P., W.A. Banks, H.L. Collins and C.E. Stringer. 1981. Large area tests of AC-217,300 bait for control of imported fire ants in Alabama, Louisiana, and Texas. Southwest Entomol. 6: 150-157.

Phorid Fly Detection Enhancement with a Modified Electric Livestock Prod

Charles L. Barr
Alejandro Calixto

Texas Cooperative Extension

The decapitating phorid fly Pseudacteon tricuspis Borgmeier has been released at a number of sites around the United States for the control of red imported fire ants (Solenopsis invicta Buren). Field detection of phorids is a tedious and often unsuccessful endeavor. The usual procedure is to mechanically disturb a fire ant mound, crush a number of ants, then simply observe the mound for the appearance of phorids. (Porter 1998). Another method used by researchers at the University of Texas is to place small piles of fire ant midden material in plastic containers or white ceramic tiles and watch for the appearance of the flies.(Gilbert and Patrock 2002). It is common to spend at least 15 minutes observing a single disturbed mound and numerous mounds must be disturbed to have a reasonable degree of confidence that flies are or are not present.

In rearing facilities at the USDA-ARS, CMAVE center in Gainesville, FL, the fly rearing boxes contain two electric plates spaced approximately 1.5 mm apart. When the plates are charged, an ant crossing the gap is "electrically stimulated" to release alarm pheromone. The result is attack stimulation by the flies and increased oviposition. (S.D. Porter, personal communication). The basis for this mechanism is that electrical stimulation causes the release of numerous semiochemicals by the ants including alarm and orientation pheromones. (Vander Meer, et al. 2002). Building on this concept, we felt that electrical stimulation of ants in the field could possibly increase the success rate of phorid fly detection so we modified a commercially available electric livestock prod for this purpose. The prod appeared so successful in day-to-day use that we conducted an experiment to test its effectiveness.

Materials and methods

The stimulation device was constructed using an electric livestock prod, TheBlueOne™ LMPlus®, manufactured by Hot Shot®. This particular model had a 30-inch wand. Modifications were made by soldering two #1 size metal paper clips to the prod's metal electrode prongs. The paper clips overlapped in a parallel manner with a spacing of approximately 3/16th-inch apart and not touching the opposite prong. Ideally, when the prod is energized, there is no electrical arc until an ant walks between the paper clips. In practice, however, there is usually an intermittent arc as the prod charges and discharges. Our most common way to use the prod, and the one used in this experiment, is to lay the tip on the mound and more-or-less continuously electrify ants.

This test was conducted at the Five Eagle Ranch, located approximately five miles north-north-east of Caldwell, Texas. Two releases of Pseudacteon tricuspis were made on the ranch in May 2002 and April 2003. Overwintered flies were first detected in April 2003 using this device. Subsequent evaluations showed there to be substantial numbers of flies in the area. The test was conducted on the afternoon of September 15, 2003. Pairs of active fire ant mounds were selected in the phorid-infested area to compare the effects of mechanical disturbance versus mechanical

plus electrical disturbance. Efforts were made to choose mounds of similar size and activity level in similar habitats; i.e. closed canopy woods, wood/pasture edge, open pasture.

At approximately the same time, each author would disturb one of a pair of mounds. One by mechanical means (usually a boot or stick) followed by the crushing of a number of ants between the fingers. The other mound was less vigorously disturbed by mechanical means, then the prod tip was placed on the mound and energized intermittently until the first phorid fly appeared. After the arrival of the first fly on each mound, that mound was observed for five minutes and the total number of flies counted. If no flies appeared on a mound, time recording was stopped after five minutes. Data were taken on the time of first fly appearance and maximum number of flies for each mound. The procedure was then repeated on another pair of mounds and so on, until a total of 11 pairs were observed. Data were analyzed using SAS PROC MEANS procedures with paired observations. Data were also analyzed using PROC TTEST with Cochran and Cox Approximation.

Results

The mean time of first fly observation for electrically stimulated mounds was 108 ($\pm$ 66) seconds versus 271 ($\pm$ 88) seconds for mechanically disturbed mounds. Probability for the paired analysis was $P = 0.0027$; t-test (df = 20), $P = 0.0001$. Flies were observed over all 11 electrically stimulated mounds, but only three mechanically stimulated mounds within five minutes of disturbance. Time to first observation, electrical, ranged from 47 to 240 seconds. Time to first observation, mechanical, ranged from 61 to 300 seconds, but only on the three mounds.

The mean number of flies per electrically stimulated mound was 5.7 ($\pm$ 2.05) and for mechanically disturbed, 0.55 ($\pm$ 1.04). Probability for the paired analysis was $P = 0.0001$; t-test (df = 20), $P = 0.0001$. Results are summarized in Table 1.

Table 1. Comparison of electrical vs mechanical stimulation of red imported fire ant mounds for the detection of *Pseudacteon tricupsis*. Mound pairs = 11. Sept. 15, 2003, Burleson County, Texas.

Parameter	Electrical	Mechanical
Mean time to first observation (sec.)	108 ± 6	271 ± 88
Mean number of flies per mound	5.7 ± 2.05	0.55 ± 1.04
Mounds with flies (max. time 340 seconds)	11 (100%)	3 (27%)
Max/min. number flies on any mound	9 / 2	3 / 0

Discussion

Results indicate that the modified electric livestock prod was highly effective at increasing the number of phorid flies observed over fire ant mounds and it greatly reduced the amount of time required to observe the first fly. Perhaps most importantly, flies were observed on 100% of the mounds where electrical stimulation was used, versus 27% where only mechanical disturbance was used. The livestock prod has also been used during *P. tricuspis* release procedures with observations indicating increased fly retention over mounds and increased

oviposition attacks.

Such behavior is typical of damage caused by red imported fire ants invading electrical equipment. (Slowik, et al. 1996; MacKay et al. 1992). Ants quickly form a large cluster around the paperclips. Flies are often seen attacking ants in close proximity to this cluster and even on the plastic part of the tip itself. Activation of the prod also seems to re-stimulate fly attack after their initial rush of attacks subsides.

Though there are undoubtedly ways in which the existing device can be improved, the model tested was inexpensive (approximately $60), quickly modified, rugged and is easily carried in vehicles and by personnel. It appears to greatly enhance the chances of success when trying to detect phorid flies in the field.

Literature Cited

Gilbert, L.E. and R.J.W. Patrock. 2002. Phorid flies for the biological suppression of imported fire ants in Texas: region specific challenges, recent advances and future prospects. SW Ento. Suppl. No. 25:7-17.

MacKay, W.P., S. Majdi, J. Irving, S.B. Vinson and C. Messer. 1992. Effect of electrical fields on the behavior of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera:Formicidae). Environ. Entomol. 21:866-870.

Porter, S.D. 1998. Host specific attraction of Pseudacteon flies (Diptera:Phoridae) to fire ant colonies in Brazil. Fla. Entomol. 81(4):423-429.

Slowik, TJ; Thorvilson, HG; Green, BL. 1996. Red imported fire ant (Hymenoptera: Formicidae) response to current and conductive material of active electrical equipmentJ. Econ. Entomol., 89(2):347-352.

Vander Meer, R.K., T.J. Slowik, H.G. Thorvilson. 2002. Semiochemicals released by electrically stimulated red imported fire ants, *Solenopsis invicta* (Hymenoptera:Formicidae). J. Chem Ecol. 28(12):2585-2600.

CONFINEMENT-TRAY COLOR AFFECTS PARASITISM RATES OF ATTACKING *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE) IN A LABORATORY REARING SYSTEM

Larry G. Thead[1] and Douglas A. Streett[2]

USDA, ARS Biological Control and Mass Rearing Research Unit, Box 5367, Mississippi State, MS 39762'
USDA, ARS Biological Control of Pests Research Unit, P. O. Box 225, Stoneville, MS 38776[2]

Abstract

Distribution of the phorid fly (Pseudacteon *curvatus* Borgmeier), a natural parasitoid of imported fire ants (Solenopsis spp.), was tested in a laboratory rearing system for dependence on background color. Ants confined within a tray with background colors (olive drab, brown or ruddy brown) were parasitized at significantly higher rates than ants confined within a white background tray. This study highlights a factor that may be influencing production levels in a phorid rearing system.

Introduction

Successful laboratory mass rearing of Pseudacteon spp. (phorid) flies that are parasitoids of imported fire ants (Solenopsis spp.) uses large attack boxes as described by Vogt (2002), and Vogt and Streett (2003). A series of white plastic trays within each attack box contains live host ants (Solenopsis richteri Forel, and Solenopsis *invicta* Buren x *richteri*), which are exposed to phorid attack for 3-4 d and then removed and held for parasitoid development. The host ants confined inside each tray are induced to trail back and forth within each tray by providing brood for them to carry, and alternately raising and lowering paired, inverted cups under which the ants seek shelter. This system helps avoid ant "freezing" and "clumping", constantly exposing them for attack. Specific climatic conditions (26°-29° C, 80-90% RH) are maintained within the attack box to minimize fly and ant mortality and to maximize attack rates. Other external factors, including light intensity, affect the distribution of attacking phorids in the attack box (Vogt, 2002). Controlling these factors helps to maximize fly distribution.

As noted by several authors (Porter 2000, Porter and Alonso 1999, Porter and Briano 2000, Folgarait et al. 2002) Pseudacteon flies are rather host specific, preferentially attacking a specific species of ant. It is postulated (Orr et al. 1997, Porter 1998) that chemical cues play a significant role in the flies' ability to locate the proper host from a distance. As the flies hover closer to the host, visual cues likely play a role in host parasitism (Porter 1998). If visual cues do indeed play a role in host attraction, the white tray, containing the exposed ants, might not optimize the flies' ability to locate and parasitize ants. In nature, an attacking fly

probably rarely encounters a white background. The white trays, routinely used inside the attack box and in the field to survey for fly establishment in areas where the flies have been released, offer an important advantage. It is simply easier to observe the attacking behavior of the minute flies against a white background. Several researchers (i.e. personal communication) have noted that when searching for phorids in areas where the flies have been released, objects such as the disturbed ant mound itself or ants placed on rocks located near a disturbed ant mound attract more flies than the white trays containing ants. The microclimate within the tray or the white background itself might interfere with the flies visual cues possibly explaining why fewer flies are attracted to the white background trays.

The aforementioned attack box system successfully rears offspring of *P. curvatus* that were originally collected from Las Flores, Buenos Aires Province, Argentina (Porter 2000). In this study, experiments were conducted to examine if the confinement-tray background color affects the distribution of flies within the attack box and the observed rates of parasitism. The purpose of this work was to help optimize use of rearing space, and ultimately increase the number of flies reared in the system.

Materials and Methods

The attack box described by Vogt (2002) for rearing *P. curvatus* at the USDA-ARS, BCMRRU, Mississippi State, MS, was used for the experiments. Light was provided by three 2-tube, 75 W, 244 cm fluorescent fixtures ("daylight" tubes) mounted above the box. Single-tube, 30 W, 91 cm fluorescent fixtures were located on each end of the attack box perpendicular to the main lights. Thirteen white plastic trays (27.5 cm W x 42 cm L x 12 cm H) were cleaned thoroughly prior to painting. To simulate the dark backgrounds encountered by *P. curvatus* in nature, three colors of aerosol paint were used in the study. Krylon® camouflage ultra flat olive drab # 8143, Krylon® camouflage ultra flat brown # 8142 and Krylon® primer ruddy brown #1317 (Krylon Products Group, The Sherwin Williams Co.). Three trays were painted with each of the colors. The inside surfaces of the trays were painted except for a 2 inch edge at the top perimeter of each tray. The painted trays were allowed to air dry for a minimum of 3 weeks. The remaining four trays were not painted. Prior to use, each was washed with detergent and water to remove any residue and was lined with Fluon® (Asahi Glass Ltd., Chadds Ford, PA) to prevent ant escape.

S. invicta x richteri colonies were collected from Oktibbeha and Noxubee Co., MS during Nov.-Dec. 2003, using the collecting and preparation procedures described by Vogt and Streett (2003). Ants were floated from the soil, and then passed through a 20-mesh sieve to separate smaller workers that are preferred by *P. curvatus*. Colonies were held in the laboratory at about 27°C under a 12:12 L: D photoperiod, and maintained with water, sugar water, and crickets (*Acheta domestica* L.). Ants from a single colony were used for each experiment.

Approximately 1.2 grams of workers (ca. 1526 ants) collected from a single colony along with approximately 1 gram of brood were placed into each of the 13 pans at the beginning of each experiment and were allowed to remain inside the attack box for 3-4d, where they were exposed to emerging *P. curvatus*. The numbers of emerging *P. curvatus* varied for each experiment, resulting in varying levels of parasitism for each experiment. This factor was not controlled because the experiment was conducted within an attack box used for routine mass rearing purposes.

The configuration of the attack box allowed for 13 trays arranged as described by Vogt (2002). For each experiment each of the four tray colors (olive drab, brown, ruddy brown, and white) were randomly assigned to each of three blocks. Blocks represented the four tray positions next to the location of the emerging flies, the four center tray positions and the four tray positions farthest from the emerging location. The thirteenth location, which was always white and not included in the analysis, was next to the enclosed side of the fly emergence box.

During each experiment, fly activity was monitored periodically by counting the number of attacking flies / 30 second interval / tray. After the ants had been exposed to attacking flies for 3-4d, the ants were removed from the attack box. Dead ants were discarded, brood were removed and the remaining live ants from each tray were placed inside individual sealed fluoned lined and ventilated Rubbermaid® servin' saver® (Newell Rubbermaid Inc. Freeport, IL) containers (19 cm L x 11.5 cm W x 5 cm H). A covered plastic petri dish (60 x 15 mm) containing cured castone dental stone (Dentsply Trubyte, York, PA) and a watered sugar impregnated Techwipe (Horizon Industries, Tyler, TX) were placed inside each container. These individual containers were held in an environmentally controlled room at (27° C, 60% RH, and 12:12 L: D photoperiod). Three times per week for ca. 34 days, the dead ants were collected and evaluated for parasitism. The numbers of ants parasitized per tray were recorded. The experiment was repeated three times using a different ant colony and a different tray randomization each time. Data for each colored tray within each block location were subjected to PROC GLM (SAS Institute Inc., 1990) to test for the effects of background tray color and distance from point of emergence (i.e. block effect). Significant effects were subjected to GLM least squares means test.

Results

The observations of attacking flies / 30 sec / tray were attempted, but the data were not analyzed due to the inherent difficulty of visually recording the attacking flies on the dark background trays versus the white background trays. Analysis of the data for the resulting parasitism for all experiments was a more precise measurement of treatment effect. Analysis indicated that the inability to control the numbers of attacking *P. curvatus* for each experiment resulted in a significant difference among experiments *(F = 7.49 P = 0.0025)*. The data were converted

to percentage parasitized per colored tray of the total parasitized per individual experiment to adjust for the experimental effect. A significant block effect $(F = 4.15, P = 0.028)$ was observed with significantly higher levels of parasitism in the four trays nearest the phorid release box. The four trays nearest the phorid release box represented 48.1% of the parasitized ants for all experiments. The middle four trays and farthest four trays from the phorid release box represented 24.3% and 27.6% of the parasitized ants for all experiments, respectively. The measurement of block color interaction was non-significant $(F = 2.06, P = 0.097)$. The background color of the trays had a significant effect $(F = 3.81, P = 0.023)$ on the level of parasitism. The dark background colored trays averaged higher levels of parasitized ants than the white background trays. None of the dark background colored tray treatments were significantly better, but all had significantly more parasitized ants within them than the white background trays (Table 1).

Table 1. Mean percent-parasitized ants per tray background color. Least Squares Means statistics.

Tray background	Mean % parasitized	Standard error	P
Olive drab	33.31	5.05	<0.0001
Brown	23.25	5.05	0.0001
Ruddy brown	32.09	5.05	<0.0001
White	11.34	5.05	0.0342

Flies attracted to the dark background trays overall parasitized approximately 2.6 fold more ants than in the white background trays. When the parasitism rates by flies attracted to individual colors were examined, the olive drab, ruddy brown and brown showed a 2.9, 2.8 and 2.1 fold increase in the parasitism rate, respectively, versus the parasitism rate in the white background trays.

Conclusions

This study demonstrates how confinement-tray background color may be influencing production of phorids in a mass rearing program. Additional studies are needed to determine if altering the background color of the trays within the attack box would result in an overall increase in rates of parasitism as compared to the current rearing system using the white background trays.

Acknowledgments

Hammond, Louisiana State University reviewed an earlier version of the manuscript and provided helpful comments.

References Cited

Folgarait, P. J., O. A. Bruzzone, R. J. W. Patrock and L. E. Gilbert. 2002. Development Rates and Host Specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of Fire Ants (Hymenoptera: Formicidae) in Argentina. J. Econ. Entomol. 95 (6): 1151-1158.

Orr, M. R., S. H. Seike, and L. E. Gilbert. 1997. Foraging ecology and patterns of diversification in dipteran parasitoids of fire ants in South Brazil. Ecol. Entomol. 22: 305-314.

Porter, S. D. 1998. Biology and behavior of *Pseudaction* decapitating flies (Diptera: Formicidae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol. 81: 292-309.

Porter, S. D. 2000. Host specificity and risk assessment of releasing the decapitating fly *Pseudacteon curvatus* as a classical biocontrol agent for imported fire ants. Biol Control 19: 35-47.

Porter, S. D., and L. E. Alonso. 1999. Host specificity of fire ant decapitating flies (Diptera: Phoridae) in laboratory oviposition test. J. Econ. Entomol. 92: 110-114.

Porter, S. D., and J. A. Briano. 2000. Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*. Fla. Entomol. 83: 422-427.

SAS Institute Inc., *SAS® Procedures Guide, Version 6, Third Edition,* Cary, NC; SAS Institute Inc., 1990. 705 pp.

Vogt, J. T. 2002. Light intensity affects distribution of attacking *Pseudacteon curvatus* (Diptera: Phoridae) in a laboratory rearing system. Imported Fire Ant Conference Proceedings: 16-20.

Vogt, J. T. and D. A. Streett. 2003. *Pseudacteon curvatus* (Diptera: Phoridae) laboratory parasitism, release and establishment in Mississippi. J. Entomol. Sci. 38(2): 317-320.

CONFINEMENT-TRAY COLOR AFFECTS PARASITISM RATES OF ATTACKING PSEUDACTEON CURVATUS (DIPTERA: PHORIDAE) IN A LABORATORY REARING SYSTEM

Larry G. Thead[1] and Douglas A. Streett[2]

USDA, ARS Biological Control and Mass Rearing Research Unit, Box 5367, Mississippi State, MS 39762[1]
USDA, ARS Biological Control of Pests Research Unit, P. O. Box 225, Stoneville, MS 38776[2]

Abstract

Distribution of the phorid fly (*Pseudacteon curvatus* Borgmeier), a natural parasitoid of imported fire ants, was tested in a laboratory rearing system for dependence on background color. Ants confined within a tray with background colors (olive drab, brown or ruddy brown) were parasitized at significantly higher rates than ants confined within a white background tray. This study highlights a factor that may be influencing production levels in a phorid rearing system.

Introduction

Successful laboratory mass rearing of *Pseudacteon* (phorid) flies that are parasitoids of imported fire ants uses large attack boxes. A series of white plastic trays within each attack box contains live host ants (*Solenopsis invicta* Buren × *richteri* Forel, the *hybrid fire ant*), which are exposed to Argentinean offspring of *Pseudacteon curvatus* flies for 3-4 d and then removed and held for parasitoid development. Specific climatic conditions (26°-29° C, 80-90% RH), uniform light intensity and 12:12 L:D photoperiod are maintained within the attack box to minimize fly and ant mortality, maximize attack rates and maximize fly distribution. *Pseudacteon* flies are rather host specific, preferentially attacking a specific species of ant. Chemical cues likely play a significant role in the flies' ability to locate the proper host from a distance. As the flies hover closer to the host, visual cues likely play a role in host parasitism. The white trays used in the attack box and in the field to survey for fly establishment, allow easier observation of the minute flies, but might not optimize the flies' ability to locate and parasitize ants. Several researchers (i.e. personal communication) have noted that when searching for phorids in areas where the flies have been released, objects such as the disturbed ant mound itself or ants placed on rocks located near to a disturbed mound attract more flies than the white trays containing ants. The microclimate within the tray or the white background itself might interfere with the flies' visual cues possibly explaining why fewer flies are attracted to the white background trays. In the confinement of the attack box, the white trays may affect parasitism rates.

Objective

Experiments were conducted to examine if the confinement-tray background color affects the distribution of flies within the attack box and the rates of parasitism observed. The purpose of this work was to help optimize use of rearing space, and ultimately increase the number of flies reared in the system.
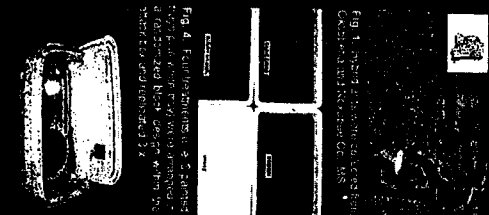
Materials and Methods

Fig 1.
Fig 4.
Fig 2.
Fig 5.
Fig 3.

Fig 7. Sub color comparison

Fig 6.

Analysis

Data were subjected to PROC GLM (SAS Institute Inc., 1990) to test for background color effects and distance from point of emergence (i. e. block effect). Significant effects were subjected to GLM least squares means test.

Results

A significant block effect ($F = 4.15$, $P = 0.028$) was observed with significantly higher levels of parasitism in the four trays nearest the phorid release box.

Fig. 9 Percentage Parasitized of Total Parasitized / Treatment

Percentage Parasitized

Olive Drab Brown Ruddy Brown White

Results (Cont.)

The four trays nearest the phorid release box represented 48.1% of the parasitized ants for all experiments. The middle four trays and farthest four trays from the phorid release box represented 24.3% and 27.6% of the parasitized ants for all experiments, respectively. The measurement of block color interaction was non-significant ($F = 2.06$, $P = 0.097$). The background color of the trays had a significant effect ($F = 3.81$, $P = 0.023$) on the level of parasitism. The dark background colored trays averaged higher levels of parasitized ants within them than the white background trays (Figure 9). None of the dark background colored tray treatments were significantly better, but all had significantly more parasitized ants within them than the white background trays. Flies attracted to the dark background trays overall parasitized approximately 2.6 fold more ants than in the white background trays. When individual colors were examined olive drab, ruddy brown and brown showed a 2.9, 2.8 and 2.1 fold increase in the parasitism rate, respectively, versus the parasitism rate in the white background trays.

Conclusions

This study demonstrates how confinement-tray background color might be influencing production of phorids in a mass rearing program. Additional studies are needed to determine if altering the background color of the ant confinement-trays within the attack box would result in an overall increase in rates of parasitism as compared to the current rates of parasitism in the rearing system using white background trays.

Myrmicinosporidium durum: A New Fire Ant Pathogen

Roberto M. Pereira

USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608

Myrmicinosporidium durum is a parasitic fungus in several ant species that produces dark, thick-walled spores, which can be seen through the insect cuticle. Seven new hosts for this fungus, all collected in the eastern United States, were recently discovered, including the red imported fire ant, *Solenopsis invicta.* Other observed hosts include: *Paratrechina vividula, Pheidole tysoni, Pheidole bicarinata, Pyramica membranifera, Solenopsis carolinensis,* and *Pogonomyrmex badius,* Fourteen other ant species from five genera were known previously as M. *durum* hosts. This is the first observation of M. *durum* in the genera *Paratrechina* and *Pyramicu.* Ants were collected from several locations in Florida, Alabama, and Tennessee using several methods including: pitfall traps, battery-operated vacuum cleaner, baited traps, and plastic tubes inserted directly into ant nests. Ant specimens were examined under dissecting scope, or light and phase microscope. Mature spores were measured and infection rates were estimated both at the colony level and at the level of local populations. Spores can be found in most ant body parts, are dark brown when mature, and clear to light brown while immature. Ants infected with mature spores appear darker than normal. Spores from different hosts were 47-57 μm in diameter. Despite similarities among the fungi found in the different hosts, definite classification of the fungi affecting different ant populations will probably require comparisons of genetic materials.

Myrmicinosporidium durum prevalence in host populations varied between 2 and 83% of the ants, and 4 to 100% of the colonies. Infection was most common in S. *carolinensis* with prevalence rates between 12 and 83%. Prevalence rates for *S. invicta* individuals were lower than for other ants, however, prevalence rates within the infected colonies were as high as 31%. In a lawn behind the USDA-ARS laboratory in Gainesville, FL, M dm-infected *S. invicta,* S. *carolinensis,* and *P. memhranifera* were collected. This is an area where residues of rejected S. *invicta* colonies have been disposed. These rejected colonies could have been infected with the fungus at the time they were discarded in the area. Because M *durum* has only now been identified from the red imported fire ant after many years of intensive research, it is possible that *S. invicta* acquired this fungus from native ants recently. This suggests the possibility of other organisms evolving into biological control agents of S. *invicta.* A relatively high prevalence of M. *durum* in *S. carolinensis* populations suggests that this thief ant species may serve as the possible inoculum source. S. *carolinensis* behavior *as* a thief ant, nesting in or near other ants and robbing food and brood from their nests, may provide opportunity for disease transmission between *S. carolinensis* and other ants.

Myrmicinosporidium durum: A New Fire Ant Pathogen

USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology

Roberto M. Pereira

Gainesville, Florida

http://cmave.usda.ufl.edu/ifahi/

ABSTRACT

Myrmicinosporidium durum, a parasitic fungus in several ant species, is reported from seven new hosts collected in the eastern United States, including *Solenopsis invicta*, *Solenopsis carolinensis*, *Paratrechina membranifera*, and *Pogonomyrmex badius*, *Pheidole tysoni*, *Pheidole bicarinata*, *Pyramica membranifera*, and *Pogonomyrmex badius*. Spores can be found in most ant body parts, are dark brown when mature, and clear to light brown while immature. Ants infected with mature spores appear darker than normal. Spores from different hosts were 47-57 μm in diameter. Prevalence in infected host populations varied between 2 and 87% of the ants, and 0.5 to 100% of the colonies. Infection was most common in *S. carolinensis* with prevalence rates between 12 and 83%. Prevalence rates for *S. invicta* individuals were lower than for other ants, however, prevalence rates within the infected colonies were as high as 31%. Observations of disease were recorded mostly from late summer and fall. Possible implications of this new disease in the red imported fire ants are discussed.

INTRODUCTION

Myrmicinosporidium durum is a parasitic fungus found infecting several ant species classified in several different subfamilies. This fungus has been reported from several localities in Europe but from only a harvester ant in the United States. The exact nature of this fungus, and the effects on the host ants remain uncertain. Because infected ants are collected in pitfall traps, the fungus effects may be minor at least until very late in its development.
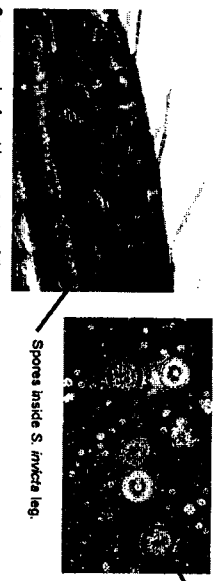
M. durum has been detected in seven new hosts including the red imported fire ant, *Solenopsis invicta*, all collected in the eastern United States. Previous surveys in the United States and South America failed to detect *M. durum* in any *Solenopsis* species.

MATERIALS and METHODS

Pitfall traps with propylene glycol were used for general ant collection. The traps were left out at least overnight, but sometimes for several days before being collected. Collected ants were preserved in 70% ethanol, and any individuals that showed signs of pathogens were examined further. A battery-operated vacuum cleaner was also used for general ant collection, and the collected material was placed in Berlese funnels for separation of arthropods. Some collections were also made with baited traps. Plastic Petri dishes (50-mm) containing a cotton pad soaked with a sugar solution left open in the field for 20-30 minutes were used in TN. Plastic vials (74 ml) capped with a punctured lid and containing a piece of Keebler's Sandies® pecan shortbread were buried in soil (10-15 cm deep), and collected after 1-3 days. Fire ants were also collected using Fluon®-coated plastic tubes inserted directly into the fire ant nests and by shoveling nest soil into Fluon®- or talc-coated 20-l plastic buckets.

Ant specimens were examined with a dissecting microscope, or with a light or phase microscope for signs of *M. durum* infection including darker coloration and the presence of round spores in different regions of the body. Mature fungal spores were measured (the longest axis and a broadest dimension perpendicular to that axis), and the average diameter and spore shape ratio were compared across ant species. Disease prevalence rates were estimated at colony and area population levels. The prevalence rates of district *S. invicta* colonies were estimated.

Southeast United States map showing locations where the ant pathogen *Myrmicinosporidium durum* was collected infecting different ant hosts.

The USDA-ARS laboratory in Gainesville FL (CMAVE – Center for Medical, Agricultural and Veterinary Entomology) and a farm in Houston Co., AL are the only locations where multiple ant species have been collected with *M. durum* infection. Infection rates where collected in FL were different in FL.

- Tennessee
- Mississippi
- Alabama
- Georgia
- South Carolina
- North Carolina
- Florida

■ Solenopsis invicta
▲ Solenopsis carolinensis
● Pyramica membranifera
✱ Pheidole bicarinata
✚ Pheidole tysoni

RESULTS

Spores can be found in most parts of the ant body, although smaller hosts do not have spores in their antennae, legs, and other regions where the development of the large spores is restricted by the body part diameter. Mature spores are dark brown while immature spores vary from a clear to light brown.

Spores inside *S. invicta* leg.

Spores removed from ant body (spores are ~ 50μm in diameter).

Spore dimensions for *Myrmicinosporidium durum* from 4 different ant hosts

Host Species	Spore diameter (μm)[a] (Mean ±SEM)	Spore shape ratio[a] (Mean ±SEM)
Solenopsis invicta	54.8 ±6.79 a	0.97 ±0.0004 ab
Solenopsis carolinensis	55.9 ±1.87 a	0.93 ±0.0004 b
Pogonomyrmex badius	47.0 ±0.24 b	0.98 ±0.0004 a
Paratrechina vividula	57.4 ±3.23 a	0.97 ±0.0001 a

[a] P-value = 2.4E-08, F = 22.32, df = 3; 36
[a] P-value = 0.0064, F = 4.99, df = 3; 36

Myrmicinosporidium durum spores in *P. badius* were approximately 16% smaller (significant at the 5% confidence level) than those in other hosts. The spores observed in *S. carolinensis* were slightly more oval than those in the other three ant hosts.

### *Myrmicinosporidium durum* prevalence rates in *Solenopsis invicta*, in eastern United States

Locality	Date	# Ants	% Prevalence	# Traps or Colonies (C)	% Prevalence
Marianna, FL - BT 23	15 Sep 2003	501	4.6	9 C	11.1
Marianna, FL - BT 24	15 Sep 2003	266	31.2	8 C	12.5
Marianna, FL - BP 55	15 Sep 2003	-	-	5 C	20.0
Marianna, FL - BT 23	7 Oct 2003	92	4.3	15 C	13.3
Marianna, FL - BT 24	7 Oct 2003	-	-	15 C	6.7
Marianna, FL - BP 55	3 Nov 2003	97	36.1	27 C	3.7
CMAVE -	Live 1581	25.9			
	Dead 97	36.1			
Gainesville, FL	14 Nov 2003	-	-	8	0.0
	19 Nov 2003	-	-	4 C	25.0
	20-23 Nov 2003	5	20.0	50	0.5
Houston Co., AL - C3	21-24 Oct 2002	41	2.4	-	-

Myrmicinosporidium durum prevalence rates in ant hosts, in eastern United States

Species	Locality	Date	# Ants	% Prevalence	# Traps or Colonies (C)	% Prevalence
Paratrechina vividula	Ames Plant., TN	Aug 2000	501	4.6	3 C	66.7
	Ames Plant., TN	Aug 2000	23	8.7	3	33.3
Pheidole tysoni	Houston Co., FL	Oct 2002	102	6.9	19	15.8
Pheidole bicarinata	Madison Co., FL	Oct 2001	6	16.7	1	100
Pogonomyrmex badius	Madison Co., FL	Aug 2003	109	23.9	3 C	100
Pyramica membranifera	CMAVE, FL	Oct-Nov 2003	Only 1 infected ant		11	9.1
Solenopsis carolinensis	Newberry, FL	Apr 2000	3	66.7	2	50.0
	Union Co., FL	Oct 2001	55	45.4	13	61.5
	Union Co., FL	Oct 2002	102	30.4	17	47.1
	Houston Co., AL	Oct 2002	151	22.5	20	25.0
	CMAVE, FL	Nov 2003	8	25.0	8	25.0

Myrmicinosporidium durum prevalence seems to be quite high in *S. carolinensis* populations. Prevalence rates for *S. invicta* populations seem to be lower than that of other ants. However, prevalence rates within the infected colonies were as high as 31%. *M. durum* prevalence was slightly higher in cadavers (36.1%) than in living ants (25.9% in a small fire ant colony collected in the field. Development of *M. durum* in host ants may cause higher mortality rates under stress.
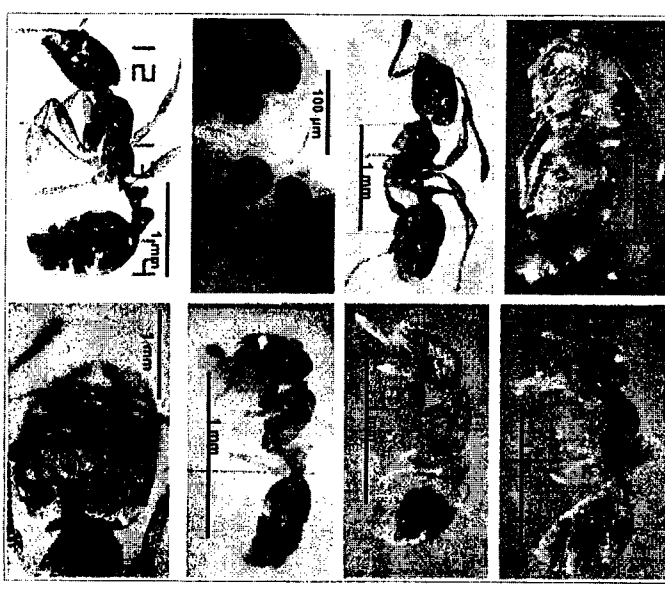
New *M. durum* ant hosts: A) *Paratrechina vividula*, B) *Pheidole tysoni*, C) *Pheidole bicarinata*, D) *Pyramica membranifera*, E) *Myrmicinosporidium durum* spores in *P. bicarinata* abdomen; F) *Solenopsis carolinensis*; G) *Solenopsis invicta*; H) *Pogonomyrmex badius* head. Note dark and light colored round spores in different body parts.

DISCUSSION

Myrmicinosporidium durum has only now been identified from *S. invicta* after many years of intensive research on this ant in numerous locations throughout the southeastern United States. Perhaps this fungus has been acquired by *S. invicta* from native ants. The relatively high prevalence of *M. durum* in *S. carolinensis* populations suggests that this species may serve as a reservoir of the disease in the region, and as a possible inoculum source for other ant populations. Because *S. carolinensis* nests in or near other ants and robs their nests, there may be ample opportunity for disease transmission between *S. carolinensis* and other ants.

A recent increase in disease detection in fire ant populations may be an indication of increased susceptibility of the North American *S. invicta* population. The predominance of less aggressive polygynous colonies may increase contact and exchange of individuals among neighboring nests, increasing disease transmission. An increase in parasitism after >70 years of unchecked expansion in the United States suggests that the imported fire ant population has acquired new diseases, perhaps from the native ants. Whether a newly acquired, or increasing disease susceptibility can slow the fire ant expansion. Exposures to increasing number of parasites, may offer hopes for containment of the fire ant population in North America.

ACKNOWLEDGMENTS

Distribution of *Thelohania solenopsae* in *Red* Imported Fire Ant Populations in Mississippi

D.A. Streett[1], Thomas Barton Freeland, Jr.[2], and Anthony M. Pranschke[1]

USDA, ARS, Biological Control of Pests Research Unit, Stoneville, MS[1]
USDA, OCE-WAOB, Delta Research and Extension Center, Stoneville, MS[2]

Abstract
A systematic survey was conducted for the microsporidian parasite, *Thelohania solenopsae* in natural populations of the red imported fire ant, *Solenopsis invicta.* A total of 151 fire ant mounds were sampled from forty-six counties in Mississippi. *T. solenopsae* was detected in 10% of the sampled mounds in this study. The intra-colony incidence of infection was generally low for infected fire ant mounds with the exception of one mound from Madison County, MS.

Introduction
Imported fire ants are an important pest in the United States with an estimated cost well over one billion dollars per year. This pest infests over 312 million acres of land in the south and southwest, and infestations are likely to spread to the north and entire west coast. The geographic range of the imported fire ant will continue to expand and increase without the implementation of a centralized pro-active regional management program. Implementation of a regional program will require an integrated pest management approach utilizing cultural, chemical and biological control strategies.

Among the biological control strategies being considered for suppressing fire ant populations are entomopathogens. One of the first entomopathogens identified from fire ants was a microsporidian parasite (Allen and Buren, 1974) that was later described as *Thelohania solenopsae* from the red imported fire ant, *Solenopsis invicta* in Brazil (Knell et al., 1977). Several other *Solenopsis* species have been reported to be infected with a similar parasite in Argentina, Uruguay, Paraguay, and Brazil. In 1998, *T. solenopsae* was discovered in field-collected *S. invicta* colonies from Florida, and was later found in *S. invicta* colonies in the Mississippi Gulf Coast area (Williams et al., 1998). The objective of this study was to determine the current distribution of *T. solenopsae* in red imported fire ant populations in Mississippi.

Materials and Methods
Samples of worker ants were collected from field colonies in Mississippi from 2001 to 2003. Mounds were mapped with a backpack Trimble 124 beacon DGPS system utilizing GIS Solo CE V3.0 software (TDS) installed on a Compaq iPAQ*. A vial sample containing 100-1000 ants was also removed from each mound, labeled for identification, and stored on ice. A sample consisting of 20 ants was removed from each vial, homogenized with a pellet homogenizer in 0.25ml of distilled water and an aliquot of the homogenate examined by phase microscopy for the presence of *T. solenopsae* spores. Colonies determined as infected were further examined for intra-colony incidence of infection. Ten ants were individually examined for each infected colony following the same protocol as our batch inspections. Spore counts at 40x magnification

were made for ten fields and averaged for each ant. Concentrations of spores per field were assigned a score: 0=none, 1=>0-1spore/field; 2= >1-5 spores/field; 3= >5 to 10 spores/field; 4= >10 spores/ field in each sample. Field data and the results on infection status were entered into ArcView 3.2a Geographic Information Systems (GIS) for the spatial presentation of the data.

Results

A total of 151 red imported fire ant mounds were sampled among 46 counties in southern and western Mississippi. Colonies of red imported fire ants were sampled as far north as Bolivar County in western Mississippi and Kemper County in eastern Mississippi. Ten percent (15 of 151) of the mounds sampled were infected with *T. solenopsae* (Fig. 1). Twenty-four percent (11 of 46) of the counties contained at least one documented infection. Nine colonies scored (1) indicating low spore counts, averaging <1 spore/field (Table 1). Only one colony, located in Madison County, scored (4), exceeding 28 spores per field. Two colonies found to have infection from the batch sample failed to produce an infection score based upon individual ant inspections.
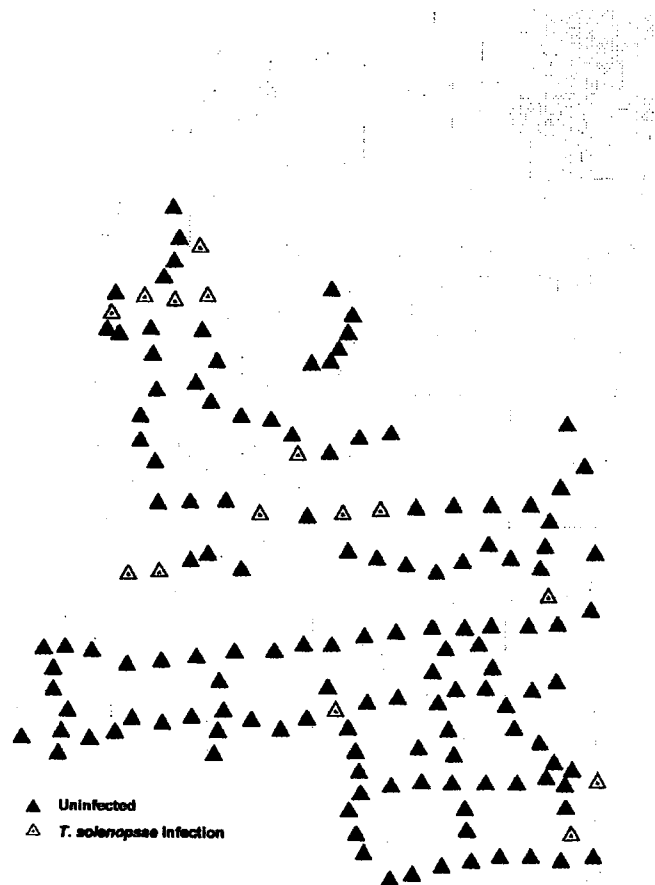

Fig. 1. Distribution of *T. solenopsae* infected Red Imported Fire Ant mounds sampled throughout Mississippi.

Table 1. Average spore counts and associated infection scores of red imported fire ant colonies sampled in Mississippi.

Colony	County	Avg. spore count/ field	Score
1	Claiborne	0.06	1
2	Claiborne	0.33	1
3	Clarke	0.31	1
4	George	1.64	2
5	Hinds	0.54	1
6	Jackson	0.21	1
7	Madison	28.7	4
8	Marion	0.42	1
9	Rankin	0	0
10	Scott	0.31	1
11	Sunflower	-	-
12	Sunflower	0	0
13	Sunflower	-	-
14	Washington	0.25	1
15	Washington	0.1	1

Conclusions

Our survey finds *T. solenopsae* infected red imported fire ant colonies in Mississippi are not limited to the Gulf Coast area. Infection levels were consistent among colonies throughout the state, with the exception of Madison County. Delineation of geographic areas containing infected colonies identifies target areas throughout the state for establishment of regional management programs. Prevalence and colony incidence of infection at these identified areas is currently under further investigation.

Acknowledgments

References Cited

Allen, G. E. and W.F. Buren. 1974. Microsporidian and fungal diseases of *Solenopsis invicta* Buren in Brazil. J. N. Y. Entomol. Soc., 32: 125-130.

Knell, J. D., G. E. Allen, and E. I. Hazard. 1977. Light and Electron Microscope Study of *Thelohania solenopsae* n. sp. (Microsporida: Protozoa) in the Red Imported Fire Ant, *Solenopsis invicta*. J. Inveretebr. Pathol., 29: 192-200.

Williams, D.F., G. J. Knue, and J.J. Becnel. 1998. Discovery of *Thelohania solenopsae* from the Red Imported Fire Ant, *Solenopsis invicta*, in the United States. J. Inveretebr. Pathol., 71: 175-176.

Distribution of *Thelohania solenopsae* in Red Imported Fire Ant Populations in Mississippi

D.A. Streett[1], Thomas Barton Freeland, Jr.[2], and Anthony M. Pranschke[1]

USDA, ARS, Biological Control of Pests Research Unit, Stoneville, MS[1]
USDA, OCE-WAOB, Delta Research and Extension Center, Stoneville, MS[2]

Abstract

A systematic survey was conducted for the microsporidian parasite, *Thelohania solenopsae* in natural populations of the red imported fire ant, *Solenopsis invicta*. A total of 151 fire ant mounds were sampled from forty-six counties in Mississippi. *T. solenopsae* was detected in 10% of the sampled mounds in this study. The intra-colony incidence of infection was generally low for infected fire ant mounds with the exception of one mound from Madison County, MS.

Introduction

Imported fire ants are an important pest in the United States with an estimated cost well over one billion dollars per year. This pest infests over 312 million acres of land in the south and southwest, and infestations are likely to spread to the north and entire west coast. The geographic range of the imported fire ant will continue to expand and increase without the implementation of a centralized pro-active regional management program. Implementation of a regional program will require an integrated pest management approach utilizing cultural, chemical and biological control strategies.

Among the biological control strategies being considered for suppressing fire ant populations are entomopathogens. One of the first entomopathogens identified from fire ants was a microsporidian parasite (Allen and Buren, 1974) that was later described as *Thelohania solenopsae* from the red imported fire ant, *Solenopsis invicta* in Brazil (Knell et al., 1977). Several other *Solenopsis* species have been reported to be infected with a similar parasite in Argentina, Uruguay, Paraguay, and Brazil. In 1996, *T. solenopsae* was discovered in field-collected *S. invicta* colonies from Florida, and was later found in *S. invicta* colonies in the Mississippi Gulf Coast area (Williams et al., 1998). The objective of this study was to determine the current distribution of *T. solenopsae* in the red imported fire ant populations in Mississippi.

Materials and Methods

Study Site: Samples of worker ants were collected from field colonies in Mississippi from 2001 to 2003. Mounds were mapped with a backpack Trimble 124 beacon DGPS system utilizing GIS Solo CE V3.0 software (TDS) installed on a Compaq IPAQ (Fig. 1). A vial sample containing 100-1000 ants was also removed from each mound, labeled for identification, and stored on ice (Fig. 2). A sample consisting of 20 ants was removed from each vial, homogenized with a pellet homogenizer in 0.25ml of distilled water and an aliquot of the homogenate examined by phase microscopy for the presence of *T. solenopsae* spores. Colonies determined as infected were further examined for intra-colony incidence of infection. Ten ants were individually examined for each infected colony following the same protocol as our batch inspections. Spore counts at 40x magnification were made for ten fields and averaged for each ant. Concentrations of spores per field were assigned a score: 0=none, 1=<10 spores/field; 2=>1-5 spores/field; 3=>5 to 10 spores/field; 4=>10 spores/field in each sample. Field data and the results on infection status were entered into ArcView 3.2a Geographic Information Systems (GIS) for the spatial presentation of the data.

Results

A total of 151 red imported fire ant mounds were sampled among 46 counties in southern and western Mississippi. Colonies of red imported fire ants were sampled as far north as Bolivar County in western Mississippi and Kemper County in eastern Mississippi. Ten percent (15 of 151) of the mounds sampled were infected with *T. solenopsae* (Fig. 3). Twenty-four percent (11 of 46) of the counties contained at least one documented infection. Nine colonies scored (1) indicating low spore counts, averaging <1 spore/field (Table 1). Only one colony, located in Madison County, scored (4), exceeding 28 spores per field. Two colonies found to have infection from the batch sample failed to produce an infection score based upon individual ant inspections.

Table 1. Average spore counts and associated infection scores of red imported fire ant colonies sampled in Mississippi

Colony	County	Avg. spore count/ field	Score
1	Claiborne	0.06	1
2	Claiborne	0.38	1
3	Clarke	0.31	1
4	George	1.64	2
5	Hinds	0.54	1
6	Jackson	0.21	1
7	Madison	28.7	4
8	Madison	0.42	1
9	Rankin	0	1
10	Scott	0.31	1
11	Sunflower	0	1
12	Sunflower	0	1
13	Sunflower	-	1
14	Washington	0.25	1
15	Washington	0.1	1

Fig. 1. Backpack Trimble 124 DGPS system

Fig. 2. Vial collection of red imported fire ants

Fig. 3. Distribution of *T. solenopsae* infected Red Imported Fire Ant mounds sampled throughout Mississippi

△ No *T. solenopsae* infection
▲ *T. solenopsae* infection

Conclusions

Our survey finds *T. solenopsae* infected red imported fire ant colonies in Mississippi are not limited to the Gulf Coast area. Infection levels were consistent among colonies throughout the state, with the exception of Madison County. Delineation of geographic areas containing infected colonies identifies target areas throughout the state for establishment of regional management programs. Prevalence and colony incidence of infection at these identified areas is currently under further investigation.

Insect diversity patterns in *Solenopsis invicta* infested areas in Oklahoma

Vedham Karpakakunjaram, Richard A. Grantham and Russell E. Wright

Department of Entomology & Plant Pathology, Oklahoma State University Stillwater, OK 74078

Abstract

The impact of Solenopsis *invicta* on native ant species and other arthropod communities has been greatly discussed during the last two to three decades, but not resolved. S. *invicta* was first reported from Oklahoma in the mid-1980s. Here, we discuss the outcome of the research conducted over a year (2003) at two sites, Adam's and Bowles' Ranches, Bryan Co., OK, as part of our Area-wide Suppression Project studies, Insect samples were collected using pitfall traps over a 48-hour period in May and September 2003. At each site, 150 acres were treated with insecticides in June 2003. At Adam's Ranch, the number of fire ants caught per pitfall trap placed in the treated area increased more than 3-fold from May to September 2003. This trend could be attributed to an increase in mound density by September 2003, though a 67% decrease was o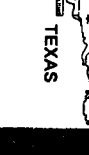bserved in mound density within 30 days of treatment. There was a 5-fold increase in the number of fire ants per trap in untreated area of Adam's Ranch, from May to September 2003, even though mound density had not changed drastically between May and September 2003. In Bowles' Ranch, the difference in the number of fire ants per trap in treated vs. untreated areas was not high. The mean morphospecies caught per trap was significantly higher in the treatment plots compared to the control plots of both sites, before insecticide treatment (Mann-Whitney U-test). Post-treatment (90-days after treatment) analyses indicated that the number of morphospecies was not significantly different between treated and control plots at Adam's Ranch. There was a significant reduction in the number of morphospecies in the treated plots of Adam's Ranch 90-days after the treatment, and the mean was not significantly different in the control plots. The treated plots at Bowles' Ranch contained significantly more number of morphospecies than the control plots in the post-treatment evaluation (Mann-Whitney U-test). Adam's and Bowles' Ranches, collectively, had 10 species of ants (excluding *S. invicta*) belonging to four different subfamilies. Amongst the morphospecies trapped at both sites, there were beetles from 14 families, flies (Diptera) from 10 families and wasps (Hymenoptera) from seven families and one superfamily. At Bowles' Ranch, the insecticide treatment had no significant effect on insect species other than RIFA, and reduction in RIFA may have actually resulted in the maintenance of species diversity. However, the results from Adam's Ranch are not conclusive due to factors like the increase in the mound density in the treatment plots by September 2003. Long-term observations would enable us to analyze the data in more detail and to understand the interspecific interactions along with the associated ecological determinants conclusively.

Insect diversity patterns in Solenopsis invicta infested ranches in Oklahoma

Vedham Karpakakunjaram, Richard A. Grantham and Russell E. Wright

Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74078

Introduction
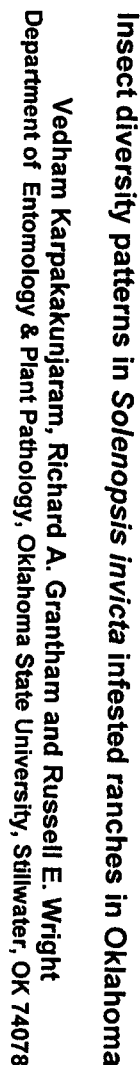
Red Imported Fire Ant, Solenopsis invicta was accidentally introduced into the United States from South America during the 1930s. Since then, the Red Imported Fire Ants (RIFA) has gradually spread and infested more than 260 million acres, from North Carolina to Texas. RIFA expanded its habitat Northward and was first reported in the mid-1980s in Oklahoma. S. invicta is more abundant in the United States than in its natural habitats in South America (Porter et al., 1992). The ecological dominance of RIFA in the introduced habitats can be attributed to the lack of natural enemies in the introduced habitats. The impact of S. invicta on native ant species and other arthropod communities has been greatly discussed during the last two to three decades, but not resolved. Here, we discuss the outcome of the research conducted from Spring to Fall 2003, at Adam's and Bowles' Ranches, Bryan Co., OK as part of our Area-wide Suppression Project studies.

Fig 1. Study Sites

TEXAS

Bowles' Ranch

Adam's Ranch

Methods
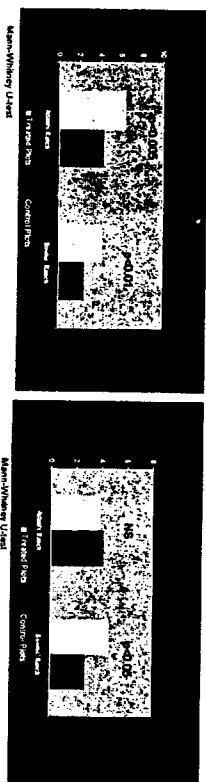
The two study sites, Adam's and Bowles' Ranches (Fig 1) have ten plots each in the 150-acre treatment area and in the peripheral control area, 9 and 12 plots, respectively. All plots are of 1/8th acre in size and divided into four quarters from the center based on the four directions. Pitfall traps were set at 14 ft and 28ft radii from the center, on all four directions, in May (Pre-treatment) and September (90-days after treatment) 2003. The traps were collected after 48 hours, sorted in the laboratory and identified. All ant samples were identified to genus/species level. Other insects and invertebrates were identified to family level and categorized as morphospecies. The four traps from the inner circle of each plot were analyzed. In June 2003, 150 acres were treated with insecticides at both sites. Data on morphospecies was statistically analyzed using Mann-Whitney U-test.

Table 1. Effect of insecticide treatment on RIFA collected in pitfall traps

Sites	PRE-TREATMENT (May 2003)				POST-TREATMENT (September 2003)			
	Treated plots		Control plots		Treated plots		Control plots	
	Mean RIFA/plot	% traps with RIFA	Mean RIFA/plot	% traps with RIFA	Mean RIFA /plot	% traps with RIFA	Mean RIFA/plot	% traps with RIFA
Adam's Ranch	7.0±7.2 n=40	90	21.1±17.19 n=40	90	22.8±39.88 n=40	90	104.11±76.02 n=36	100
Bowles' Ranch	13.7±26.17 n=36	70	60.1±78.98 n=36	100	13.4±18.11 n=40	60	144.33±164.53 n=44	100

Mean values are expressed as mean±SD

Mann-Whitney U-test

Mann-Whitney U-test

Results and Discussion

Before treatment, the mean number of S. invicta per plot was lesser in the treatment plots within the treatment area compared to the control plots (7.0 and 13.7 in the treatment plots and 21.1 and 61.5 in the control plots of Adam's and Bowles' Ranches, respectively (Table 1). Similarly, 90-days after treatment, the mean number of RIFA per plot was very low in treated plots of Adam's and Bowles' Ranch compared to the control plots in the respective sites. However, in almost all these cases, high number of RIFA were contributed by fewer traps which may have resulted in very high standard deviation values (Table 1).

The mean morphospecies caught per trap was significantly higher in the treatment plots compared to the control plots of both sites, before insecticide treatment (Fig 2; Mann-Whitney U-test: Adam's Ranch at p<0.005 and Bowles' Ranch at p<0.01). Post-treatment (90-days after treatment) analyses indicated that the number of morphospecies was not significantly different between treated and control plots at Adam's Ranch (Fig 3; Mann-Whitney U-test: at p>0.05). There was a significant reduction in the number of morphospecies in the treated plots of Adam's Ranch 90-days after the treatment, while the mean remained relatively unchanged in the control plots. This may have caused the differences in the number of morphospecies between treated and control plots to be insignificant in the September samples. The treated plots at Bowles' Ranch, contained significantly more number of morphospecies than the control plots in the post-treatment evaluation (Fig 3; Mann-Whitney U-test at p<0.05).

Adam's and Bowles' Ranches, collectively, had 10 species of ants (excluding S. invicta) belonging to four different subfamilies (Table 2). Crematogaster sp. Pogonomyrmex sp. and Neivamyrmex sp. were found in one trap each, while the other species were trapped more frequently. Amongst the morphospecies trapped at both sites, coleopterans were the most common group of insect (Table 2). Beetles from 14 families, flies (Diptera) from 10 families and wasps (Hymenoptera) from seven families and one superfamily were collected from Adam's and Bowles' Ranches.

Conclusions

Results from Bowles' Ranch indicate that insecticide treatment has no significant effect on insect species other than RIFA, and that reduction in RIFA may actually result in the maintenance of species diversity. However, the results from Adam's Ranch are not conclusive due to many factors, the increase in the mound density in the treatment plots by September 2003 being a major one. Long-term observations would enable us to analyze the data in more detail and to understand the interspecific interactions along with the associated ecological determinants conclusively.

References

1. Porter, S.D., Fowler, H.G., and MacKay, W.P., 1992. J. Econ. Entomol. 85, 1154-1161.

Table 2. List of ant species identified from the pitfall traps

Order	Family	Subfamily and Species
Hymenoptera	Formicidae	**Ponerinae:** Ponera trigona opacior
		Myrmiinae: Crematogaster sp. Monomorium minimum Pheidole sp. Pogonomyrmex sp. Neivamyrmex sp. Solenopsis molesta
		Formicinae: Paratrechina sp.
		Dolichoderinae: Tapinoma sp. Forelius sp.

Table 3. List of morphospecies trapped in the pitfall traps

Order	Family
Coleoptera	Carabidae, Scarabaeidae, Histeridae, Curculionidae, Chrysomelidae, Cleridae, Anthicidae, Anobiidae, Elateridae, Staphylinidae, Rhizophagidae, Phalacridae, Cryptophagidae, Scymaenidae.
Diptera	Calliphoridae, Cecidomyiidae, Chironomidae, Dolichopodidae, Drosophilidae, Muscidae, Sepsidae, Stratiomyidae (pupae), Tachinidae, Tipulidae.
Hymenoptera	Chalcoidea (Superfamily), Culicidae, Diapriidae, Eumenidae, Ichneumonidae, Pompilidae, Scelionidae, Tiphiidae (Subfamily – Methochinae).
Homoptera	Aphididae, Cicadellidae, Delphacidae
Hemiptera	Anthocoridae, Cydnidae, Lygaeidae, Pyrrhocoridae.
Orthoptera	Acrididae, Gryllidae, Gryllacrididae.
Other insect groups	Blattaria – Blattidae, Thysanoptera – Terebrantia (Suborder), Collembola – Symphypleona and Arthropleona (Suborders), Diplura – Japygidae (Suborder), Protura, Lepidoptera – microlepidopteran moth.
Other invertebrates: Araneae, Acari, Diplopoda, Isopoda, Mollusca and Annelida.	

A Downsized Rearing System for Phorid Flies

Lee Eisenberg
Dept. of Entomology,
404 Life Sciences Building,
Louisiana State University Agricultural Center,
Baton Rouge, LA, 70803

Abstract

An inexpensive rearing system for Phorid Flies (*Diptera: Pseudacteon)* on a small scale is reported that uses a bakery "proofer" (used to raise yeasted breads) as a primary source of humidification, and a commercial incubator piped to a household ultrasonic humidifier to maintain the high levels of relative humidity necessary for successful development and emergence. The primary elements- the racks of resident colonies, parasitized colony fragments, pupal incubator, attack box and proofer-occupy approximately 200 sq.ft. of floor space. A pupal watering regime that may decrease emergence time is also reported.

A Downsized Phorid fly Rearing System

By Lee Eisenberg, Seth Johnson and Don Henne
Dept of Entomology,
LSU Ag Center
Baton Rouge, LA 70803

ABSTRACT

An inexpensive rearing system for Phorid flies (Diptera: *Pseudacteon*) on a small scale is reported that uses a Bakery "proofer" (used to raise yeasted breads) as a primary source of humidification, and a incubation piped to a household ultrasonic humidifier to maintain the high levels of humidity necessary for pupal development and emergence. The primary elements- the racks of resident colonies, parasitized colony fragments, pupal incubator, attack box and proofer- occupy approximately 200 sq. ft of floor space.

INTRODUCTION:

Parasitoids of the genus *Pseudacteon* have been released as a classical biocontrol measure against the imported fire ant, *Solenopsis invicta*, which has infested 300 million acres since its introduction to North America 62 years ago. The flies have become established and are dispersing in the wild, where they are expected to exert top-down pressure analogous to that in its native Brazil.

REARING SYSTEMS

Pseudacteon tricuspis was first reared by Porter (2000) in screened attack boxes in rooms held at Gainesville at 80-90%R.H. and 28-32°C. The rearing facility in Gainesville is an expanded version of this approach, with humidity supplied by commercial humidifiers in large rooms housing up to eight attack boxes.

Temperature is controlled by fluorescent and infrared lights, space heaters, and commercial space heaters.

Vogt et al. (2003) introduced enclosed attack boxes modeled on nesting boxes used by ARS personnel. Air mixed with steam from a central boiler is drawn from a mixing room by blowers to provide optimal humidity to a series of attack boxes. Both humidity and temperature are controlled by a combination of sensors and timers. Timers also regulate litter cups, which raise and drop to disrupt the ants, and an access door that opens to allow entry of the phorids at times of peak activity. Both are operated pneumatically.

S. D. Porter has since employed an attack box with a humidifier located in a chamber in the base (personal communication).

Under construction

Loading the attack box

Pneumatic controls with stamp computer

Interior of the proofer
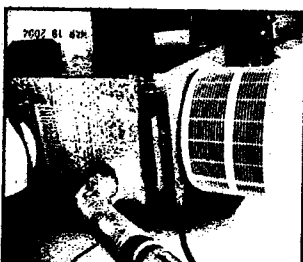
The proofer & air cleaner in the colony room

The attack box

Working with the ants

Sieving out ants

Feeding out ants

At LSU, our rearing system was modeled after Vogt et al. (2003) with some changes to accommodate a smaller work area that was continuous with office space and adjacent to a variety of other research projects. Central steam was not a viable means of supplying humidity, as LSU's central steam system contains difficult-to-remove amines that serve to reduce corrosion.

A stainless steel bakery proofer, (designed to raise yeasted breads) was converted to an "amined plenum" by removing the door and fitting a 2x3 ft. piece of sheet metal in its place. The sheet metal has two openings that accept a 4" duct and a grill that allows regulation of the airflow into the proofer. The duct pipe leads to a blower that supplies the attack box. Within the proofer are two heating elements, one of which surrounds a basin fed by small reservoir of water, and stainless steel shelves that accept 18 x 13" sheet pans. The shelves were removed and used to hold pupal trays in the emergence box at the end of the habitat.

The proofer draws pre-humidified air from the colony room (10 x10') which is maintained at 28°C and ≈ 76% relative humidity with space heaters and a large household humidifier (Grainger, etc.). Warm moist air (≈40°C, 96% R.H.) rises positively through the ductwork and diffuses across the attack box with only occasional heater assistance. Temperature is controlled by a combination of fluorescent and infrared lights. A household humidifier in front of the proofer's air intake provides for accelerated humidification when the box has been open. A Stamp microcomputer (Parallax) further regulates temperature, humidity, and the litter cup assembly.

A sink in the colony room provides water, and counter space holds a compressor to power the pneumatic system, the large humidifier, and heaters. Racks holding trays of ant colonies are lined up against one wall. An air cleaner provides air circulation, and helps to reduce fungal growth.

Ants are floated out of Teflon-lined buckets in a deep sink by allowing water to drip until the ants mass on the side of the bucket ahead of the rising water. They are then removed with a bowl scraper and placed in an 18 x 26" plastic food storage container on a bakery rack in the colony room. The ants are sieved to remove undesirable morphs and placed in the attack box to serve as hosts for the phorids.

Once exposed to attack, the colony sub-fragments are held on a cart in the colony room in 11 x7" plastic food storage containers (Ziploc, WalMart) supplied with water tube, sugar water, and a large humidity block (3 x 4") made of dental plaster (Denstone, J&B Dental Supply) to maintain ≥ 93% R.H. required for phorid development. After decapitation, (beginning ≈ 10 days after parasitization) the contents of the middens pile are aspirated and placed on plaster trays and held in a commercial incubator until rearing emergence. They are then placed in the emergence box, so that they can attack and parasitize fire ants and begin the cycle again

REFERENCES:

Porter, S.D. 2000. Host specificity and risk assessment of releasing the decapitating fly *Pseudacteon curvatus* as a classic biocontrol agent for imported fire ants. Biol Control 19: 35-47.

Vogt, J.T., S.D. Porter, D.A. Nordlund and R. Smith. 2003. A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. Biol. Control 28: 346-353.

Release of *Pseudacteon curvatus* into Black and Hybrid Imported Fire Ant Populations in Northern Alabama, Southern Tennessee and Eastern Mississippi

Ken E. Ward[1], L.C. 'Fudd' Graham[2], Larry G. Thead[3],
Jason B. Oliver[4], Rufina N. Ward[1] and James T. Vogt[5]

[1]Department of Plant and Soil Science, PO Box 1208,
Alabama A&M University, Normal, AL 35762

[2]Department of Entomology and Plant Pathology, 301 Funchess Hall,
Auburn University, Auburn AL 36849

[3]USDA, ARS Biological Control and Mass Rearing Research Unit,
Box 5367, Mississippi State, MS 39762

[4]Tennessee State University Institute for Agricultural and Environmental Research
TSU Otis L. Floyd Nursery Research Center, 472 Cadillac Lane,
McMinnville, TN 37110

[5]USDA, ARS Biological Control of Pests Research Unit, PO Box 67,
Stoneville, MS 38776

Introduction

The black imported fire ant (BIFA), Solenopsis richteri, occupies about 30,000 km^2 in northeastern MS and northwestern AL. A broad band of hybridization between BIFA and the red imported fire ant (RIFA), *Solenopsis invicta,* occurs from the Mississippi River to Atlanta GA, occupying ca. 130,000 km^2 (Shoemaker et al. 1994). Given the amount of area occupied by BIFA/hybrids, they undoubtedly have a major economic impact as pests in the southeastern U.S. Biological control agents that can become established and perpetuate themselves naturally are promising as long-term agents of control for imported fire ants, especially in areas where the use of insecticides is not economically practical. The introduction and establishment of such natural enemies of imported fire ants from South America has been a major effort of USDA-ARS in recent years. Among the most promising organisms for biological control of fire ants are several species of endoparasitic phorid flies in the genus *Pseudacteon* that produce larvae that decapitate worker ants and pupate inside their empty heads (Porter 1998). A biotype of the species Pseudacteon *curvatus,* derived from Las Flores, Buenos Aires Province, Argentina has been shown to strongly prefer its natural host, S. richteri, as well as BIFA and BIFA/RIFA hybrids from the U.S. (Porter and Briano 2000). P. *curvatus* derived from this biotype are being reared in USDA-ARS labs in Gainesville, FL and Starkville, MS.

We report here an ongoing regional cooperative effort among personnel at USDA, ARS BCPRU and BCMRRU, Alabama A&M University, Auburn University and Tennessee State University to release *P. curvatus* in black and hybrid imported fire ant populations. Our objectives are to: (1) establish phorids in several paired release/control sites in northern AL, southern TN and eastern MS; (2) follow their geographic spread; and (3) statistically assess phorid impact on fire ant populations and native ant fauna.

Methods

Release sites

Release/control sites with similar fire ant population densities were located in the following areas (release dates in parentheses):

Alabama- Madison/Limestone counties (July 2002); Tishomingo (MS)/Colbert (AL) counties (June 2003); Walker County (August 2002); Cullman County (May 2003)

Tennessee- Franklin/Giles counties (September 2002); Franklin/Giles counties (different release site, September 2003)

Mississippi- Clay County (Knox site, May 2002); Clay County (Prima site release 1, August 2002; Prima site release 2, May 2003). For more detail, see Vogt and Streett (2003).

Release/control sites were fescue/clover cow pastures lightly to moderately grazed; water and wooded areas were present in or near sites.

Release protocol and detection of phorids

Six fire ant mounds, centrally located within the release site, were collected weekly for four weeks and transported to the phorid rearing facility (USDA-ARS, Mississippi State, MS) for exposure to phorids (50 adult flies/colony/day). Colonies with parasitized workers were then returned to the release site and placed back into mound/colony remnants from which they were originally collected. In most cases the first check for presence of phorids was made approximately 45 days after the last release. To monitor for flies in the field, white boxes (28 x 42 x 12 cm deep) were placed on the ground near mounds and about 1 gram of worker ants were added to each box; a number of ants were then crushed within each box. Alternative methods were also tested, including mound disruption with rocks (ants crushed on rock surface) and mixing of worker ants from different mounds within small boxes. Boxes were then periodically observed for the presence of phorids. Once flies were detected consistently observations were expanded beyond the release site to monitor movement of flies into new areas. Release and control sites and phorid population expansions were georeferenced using a Starlink Invicta DGPS receiver (Tripod Data Systems, Corvallis, OR). Observed phorids were counted and some collected as vouchers.

Phorid Impacts on fire ants and native ants

In order to measure phorid impact on fire ant populations, fire ant densities in control and release sites were estimated, using five 3 x 100 m belt transects per site. Estimates were made before and after phorid establishment. Degree of fire ant dominance of control and release sites was estimated by placement of hot dog baits along the belt transects (10 per transect at 10 m intervals) used to estimate fire ant densities, before and after phorid establishment. Finally, native ant species diversity, before and after phorid establishment, was estimated for control and release sites, using pitfall traps (9 dram plastic vials, 2.56 cm diameter) placed along belt transects. Representatives of ant species and other arthropods collected were kept as voucher specimens.

Results

Phorid Detection

Alabama-Thus far, phorids have been detected at 3 of 4 Alabama release sites (Madison, July 2003; Walker, August 2003; Cullman, August 2003). The fourth site (Tishomingo), along the Natchez trace at the AL/MS line, has not yet been checked. Phorid populations have not been detected beyond release sites for any of these areas.

Tennessee- Phorids have been detected at the first Franklin County site (July 2003); the second Franklin county site has not been checked. Phorids have not been detected beyond the release site.

Mississippi- Phorids have been detected at both the Knox (July 2002) and Prima release 2 (August 2003) sites in Clay County. By fall 2002 field-reared flies had been recovered 600 m from the Knox release site; by spring 2003 flies were recovered as far as 2 km from the Knox release area.

Phorid Impacts

There has not yet been sufficient time or buildup of phorid populations to detect impacts on fire ant or native ant populations.

Conclusions and Future

Detection of field-reared phorids at 6 of 8 release sites is encouraging; there is good reason to believe that flies will be detected at the other two sites during spring/summer 2004. We will continue to monitor for phorid presence and spread and will collect fire ant density and native ant diversity data twice annually in subsequent years to document phorid fly impacts, if any. Additional releases may be made, pending funding.

Literature Cited

Porter, S.D. 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera; Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol. 88: 1-18.

Porter, S.D. and J.A. Briano. 2000. Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*. Fla. Entomol. 83: 422-427.

Shoemaker, D.D., Ross, K.G. and M.L. Arnold. 1994. Development of RAPD markers on two introduced fire ants, *Solenopsis invicta* and *Solenopsis richteri*, and their application to the study of a hybrid zone. Mol. Ecol. 3: 531-539.

Vogt, J.T. and D.A. Streett. 2003. Note. *Pseudacteon curvatus* (Diptera: Phoridae) laboratory parasitism, release and establishment in Mississippi. J. Entomol. Sci. 38: 317-320.

Release of *Pseudacteon curvatus* into Black and Hybrid Imported Fire Ant Populations in Northern Alabama, Southern Tennessee and Eastern Mississippi

Kea E. Ward[1], L.C. "Fudd" Graham[1], Larry G. Thead[2], Jason B. Oliver[3], Rufina N. Ward[4] and James T. Vogt[5]

[1]Department of Plant and Soil Science, PO Box 1208, Alabama A&M University, Normal, AL 35762; [2]Department of Entomology and Plant Pathology, 301 Funchess Hall, Auburn University, Auburn AL 36849; [3]USDA, ARS Biological Control and Mass Rearing Research Unit, Box 5367, Mississippi State, MS 39762; [4]Tennessee State University Institute for Agricultural and Environmental Research, TSU Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110; [5]USDA, ARS Biological Control of Pests Research Unit, PO Box 67, Stoneville, MS 38776

INTRODUCTION

The black imported fire ant (BIFA), *Solenopsis richteri* occupies about 30,000 km² in northeastern MS and northwestern AL. A broad band of hybridization between BIFA and the red imported fire ant (RIFA), *Solenopsis invicta*, occurs from the Mississippi River to Atlanta GA, occupying ca. 130,000 km² (Shoemaker et al. 1994). Given the amount of area occupied by BIFA/hybrids, they undoubtedly have a major economic impact as pests in the southeastern U.S. Biological control agents that can become established and perpetuate themselves naturally are promising as long-term agents of control for imported fire ants, especially in areas where the use of insecticides is not economically practical. The introduction and establishment of such natural enemies of imported fire ants from South America has been a major effort of USDA-ARS in recent years. Among the most promising organisms for biological control of fire ants are several species of endoparasitic phorid flies in the genus *Pseudacteon* that produce larvae that decapitate worker ants and pupate inside their empty heads (Porter 1998). A biotype of the species *Pseudacteon curvatus* strongly prefer its natural host, *S. richteri*, as well as BIFA and BIFA/RIFA hybrids from the U.S. (Porter and Briano 2000). *P. curvatus* derived from this biotype are being reared in USDA-ARS labs in Gainesville, FL and Starkville, MS.

We report here an ongoing regional cooperative effort among personnel at USDA, ARS BCPRU and BCMRRU, Alabama A&M University, Auburn University and Tennessee State University to release *P. curvatus* in black and hybrid imported fire ant populations. Our objectives are to: (1) establish phorids in several imported fire ant populations, (2) follow their geographic spread; and (3) statistically assess phorid impact on fire ant populations and native ant fauna.

Pseudacteon curvatus, adult female

Release Sites

METHODS

Release sites:

Release/control sites with similar fire ant population densities were located in the following areas (release dates in parentheses):

Alabama– Madison/Limestone counties (July 2002); Tishomingo (MS)/Colbert (AL) counties (June 2003); Walker County (August 2002); Cullman County (May 2003)

Tennessee- Franklin/Giles counties (September 2002); Franklin/Giles counties (different release site, September 2003)

Mississippi: Clay County (Knox site, May 2002); Clay County (Prima site release 1, August 2002; Prima site release 2, May 2003). For more detail, see Vogt and Streett (2003).

Release/control sites were fescue/clover cow pastures lightly to moderately grazed; water and wooded areas were present in or near sites.

Release protocol and detection of phorids:

Six fire ant mounds, centrally located within the release site, were collected weekly for four weeks and transported to the phorid rearing facility (USDA-ARS, Mississippi State, MS) for exposure to phorids (350 adult flies/colony/day; see Vogt et al., 1993 for more information). Colonies with parasitized workers were then returned to the release site and placed back into mound/colony remnants from which they were originally collected. In most cases, the first check for presence of phorids was made approximately 45 days after the last release. To monitor for flies in the field, white boxes (28 x 42 x 12 cm deep) were placed on the ground near mounds and about 1 gram of worker ants were added to each box; a number of ants were then crushed within each box. Alternative methods were also tested, including mound disruption with rocks (ants crushed on rock surface) and mixing of worker ants from different mounds within small boxes. Boxes were then periodically observed for the presence of phorids. Once flies were detected consistently, observations were expanded beyond the release site to monitor movement of flies into new areas. Release and control sites and phorid populations were georeferenced using a Starlink Invicta DGPS receiver (Tripod Data Systems, Corvallis, OR). Observed phorids were counted and some collected as vouchers.

Phorid impact on fire ants and native ants:

In order to measure phorid impact on fire ant populations, fire ant densities in control and release sites were estimated, using five 3 x 100 m belt transects per site. Estimates were made before and after phorid establishment. Degree of fire ant dominance of control and release sites was estimated by placement of hot dog baits along the belt transects (10 per transect at 10 m intervals) and release sites for any of these areas, before and after phorid establishment. Finally, native ant species diversity, before and after phorid establishment, was estimated for control and release sites, using pitfall traps (9 dram plastic vials, 2.56 cm diameter, 10 per transect at 10 m intervals) placed along belt transects. Representatives of ant species and other arthropods collected were kept as voucher specimens.

Phorid Detection:

Alabama–Thus far, phorids have been detected at 3 of 4 Alabama release sites (Madison Co., July 2003; Walker Co., August 2003; Cullman Co., August 2003). The fourth site (Tishomingo Co.), along the Natchez trace at the AL MS line, has not yet been checked. Phorid populations have not been detected beyond release sites for any of these areas.

Tennessee- Phorids have been detected at the first Franklin Co. site (July 2003); the second Franklin Co. site has not been checked. Phorids have not been detected beyond the release site.

Mississippi- Phorids have been detected at both the Knox (July 2002) and Prima release 2 (August 2003) sites in Clay Co. By fall 2002 field-reared flies had been recovered 600 m from the Knox release site; by spring 2003 flies were recovered as far as 2 km from the Knox release site.

Phorid Impacts:

Phorid populations in our release areas are still too small to have detectable impacts on resident fire ant populations.
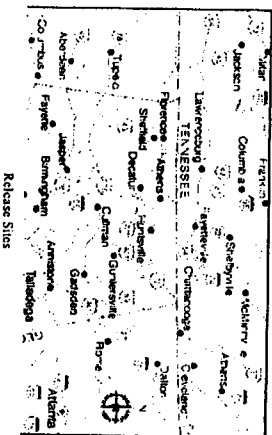
RESULTS

CONCLUSIONS AND FUTURE

Detection of field-reared phorids at 6 of 8 release sites is encouraging; there is good reason to believe that flies will be detected at the other two sites during spring/summer 2004. We will continue to monitor for phorid presence and spread and will collect fire ant density and native ant diversity data twice annually in subsequent years to document phorid fly impacts, if any. Additional releases may be made, pending funding.

LITERATURE CITED

Porter, S.D. 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol. 88:1-18.

Porter, S.D. and J.A. Briano 2000. Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*. Fla. Entomol. 83:422-427.

Shoemaker, D.D., Ross, K.G. and M.L. Arnold. 1994. Development of RAPD markers on two introduced fire ants, *Solenopsis invicta* and *Solenopsis richteri*, and their application to the study of a hybrid zone. Mol. Ecol. 3: 531-539.

Vogt, J.T. and D.A. Streett 2003. Note *Pseudacteon curvatus* (Diptera: Phoridae) laboratory parasitism, release and establishment in Mississippi. J. Entomol. Sci. 38: 317-320.

Vogt, J.T., S.D. Porter, D.A. Nordlund and R. Smith 1993. A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. Biological Control 28: 346-353.

Host/Parasite Relationship Between Red Imported Fire Ant and a Microsporidian Parasite

M. Walker Hale **and S.B. Vinson PhD**
Department of Entomology, TAMU
College Station, Texas 77843

Abstract: Thelohania solenopsae Knell, Allen and Hazard, an obligate intracellular parasite of *Solenopsis invicta* Buren, has been examined as a potential biological control mechanism to the imported fire ant. This study was conducted in 2002 and 2003 to assess the effects of *T. solenopsae* on polygynous, red imported fire ant colonies. Fiild colonies were excavated fmm College Station. TX (n=29). Brood masses of 29 colonies were measured and total number of queens per colony counted. Queens (n=263) wen dissected to determine insemination status, macerated and screened for Thelohania using phase-contrast microscopy at 400X.. There was a significant positive correlation of total queens to brood mass (p=.05) Spearmann's rho. Total queen numbers were greater in infected colonies when compared to uninfected colonies (p<.001) Mann-Whitney U . In a separate assay, infected standardized colonies (n=16) accepted more intercolonial queens (p=.002) Pearson's correlation, than uninfected standardiied colonies. An average of 67% of all queens sampled in infected mounds showed no indication of microsporidian infection, and a mean of 79% of all infected queens sampled were also inseminated. These data suggests increased acceptance of queens in an infected colony, a slow infection transfer rate within an infected mound, and behavior to transfer the intracellular parasite to inseminated queens over virgin dealates within an infected colony.

Introduction

Solenopsis invicta Buren, the red imported fire ant, an exotic intmduced species has become a serious medical and agricultural pest in the southeastern United States (Lofgren. 1986). In the last few decades, surveys have been undertaken to locate biological control organisms in the southern United States and South America (Jouvenaz,1999) to control the RIFA. One candidate for possible biological control, and bio-pesticide utility is Thelohania *solenopsae* (Microspora: Thelohaniidae) Knell Allen and Hazard. This microsporidium is an intracellular parasite of the Solenopsis complex, and principally infects multiple queen RIFA mounds. Social life not only provides various benefits, but also entails costs such as increased vulnerability to pathogens and parasites with short cydes. Pathogens and parasites might be a factor in shaping different components of the social structure of insect colonies (Keller, 1995). Specifically, pathogens might be one of the factors accounting for the association between queen number per colony and the presence/absence of polymorphic workers in ants (Frumhoff and Ward 1992; Keller. 1995). Williams et al. (1999) reported brood production was significantly reduced and queens died prematurely in laboratory infected monogyne colonies. Oi and Williams (2002) reported brood levels in polygyne infected colonies declined to 0 after 26-52 weeks. even with fertilized uninfected queens present. It is known that any RIFA colony depends on 4th instar larval forms for ingestion and regurgitation of solid foods and secretions (Petralia and Vinson, 1978). Since infection negatively affects brood number, other ways may be sought by the colony to compensate for brood loss. These experiments were conducted to elucidate possible effects of Thelohania on the colony in field and to hypothesize adaptive behavior of the RIFA.
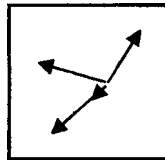
Fig. 1 Picture of free spores under 400X phase contrast microscope

abdomen of queen RIFA

Methods and Materials

All colonies for these experiments were dug from Brazos County in Spring of 2002 and 2003. Colonies were dripped out of soil using a method developed by Banks et al.(1981). Worker ants were then sampled ~25, macerated and screened for presence of Thelohania solenopsae with wet mount slides under 400X phase contrast light microscopy (Fig. I) and cmss checked using modified trichrome stain (Weber et al.,1992) to detect for meiospores or free spores. Brood masses when available were weighed. Dealated queens were given discreet numbers, counted and then frozen. Queens were then assessed for fertilization by the presence or absence of the pearl-white ovary (Fig. 2). The queens were then macerated using a Mini-Beater® and .05mm size beads. The homogenate was screened for free spores. Data were analyzed using SPSS ANOVA between infected and uninfected colonies.

A second experiment was performed to assess the disposition of queen adoption among polygyne RIFA. Colonies that had been assessed were knocked down using CO_2 then standardized to five queens, two grams of brood. and two grams of workers per replication. All colonies were fed crickets and honey water ad libitum. These replications were allowed to settle for two weeks, then five queens from different uninfected laboratory colonies were introduced to assess inter-colonial acceptance of queens on the basis of infection. Total queen numbers were recorded at 24 hours. 48 hours and 72 hours. The data was analyzed using SPSS ANOVA

Conclusions:

Brood mass is significantly and positively correlated with the number of queens in colony. Infected colonies show a decrease in brood production compared to healthy colonies.

Fig. 3 Brood mass in grams correlated to number of queens (green indicates Thelohania infected)

Evidence shows the host colony response in a red imported fire ant mound is to increase potential new queen acceptance, when infected with Thelohania.

Fig. 4 Comparison of total queen number between lines in Thelohania infected

Fig. 5 Total number of queens alive after 72 hours

In an infected mound, the majority of queens in the study remained uninfected. and a higher ratio of infected dealates are found in inseminated queens
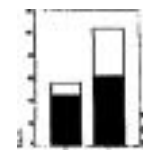

Fig. 6 Column 1 virgin dealates, column 2 inseminated dealates, pooled from infected mounds. Green indicates rate of infected in each category

Literature Cited

Banks. W.A.. C.S. Lofgren, D.P. Jouvenaz, C.E. Stringer, P.M. Bishop. D.F. Williams, D.P. Wojck, and B.M. Glancey. 1961. Techniques for collecting, rearing and handling imported fire ants. USDA. Sci. and Educ. Admin. Adv. In Agric Tech. AAT-S-21.

Frumhoff, P.C., and P.S. Ward. 1992. Individual-level selection, colony-level selection, and the association between polygyny and worker monomorphism in ants. American Naturalist 139: 554590.

Jouvenaz, D.P. 1963. Natural enemies of fire ants. Fla. Entomol. 66, 111-121.

Keller, L. 1995. Parasites, worker polymorphism, and queen number in social insects. American Naturalist. 145 (5): 842-847.

Lofgren, C.S. 1986. The economic importance and control of the imported fire ants in the United States. In Economic Impact and Control of Social insects" (S.B. Vinson, Ed.) pp. 227-256. Praeger.

Oi, D.H. And D.F. Williams. 2002. Impact of Thelohania *solenopsae* (Mircosporidia: Thelohaniidae) on polygyne colonies of red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 95 (3): 558-562.

Petralia, R.S., and Vinson, S.B. 1978. Feeding in the larvae of the imported fire ant, *Solenopsis invicta*: behavior and morphological adaptations. Am. Entomol. Soc. Am. 71: 643548.

Weber R., R.T. Bryan. R.L. Owen, C.M. Wilcox, L.G. Gorelkin, G.S. Visvesvara. 1992. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. The New Engl. Jour. Of Med. 326(3): 161-166.

Williams, D.F.. D.H. Oi and G.J. Knue.. 1989. Discovery of Thelohania solenopsae from the red imported fire ant Solenopsis invicta, in the United States. J. Invertebr. Pathol. 71: 175-176.

PCR - based analysis of spores isolated from smears by Laser Pressure Catapult microdissection techniques confirms genetic identity of spore morphotypes of Thelohania solenopsae.

Yuliya Y. Sokolova[1,3], Lacey R. McNally[2] & James R. Fuxa[1]

1 - Department of Entomology, Louisiana State Uuniversity AgCenter; 2 – Department of Pathobiological Science, School of Veterinary Medicine, Louisiana State University,
Baton Rouge, LA; 3 - Laboratory for Microbiological Control, All-Russian Institute for Plant Protection, Pobeľskogo-3, St.-Petersburg-Pushkin, Russia 899620

INTRODUCTION

We present a new approach using a position ablative laser microbeam (P.A.L.M.) microscope (Carl Zeiss, New York), equipped with UV-laser microbeam for microdissection and laser-pressure catapulting (LPC) systems, which allowed us to isolate individual *Thelohania solenopsae* (*T.s.*) spores from routinely fixed and stained smears of infected red imported fire ant. *Solenopsis invicta* (RIFA). This method was coupled with PCR to demonstrate the genetic identity of spore types in the life cycle of *T.s.*

GOALS

❑ to demonstrate the feasibility of the LPC approach for identification of microsporidia:

❑ to PCR amplify a 16S ribosome nucleotide sequence from octospores and megaspores for nucleotide sequencing;

❑ to determine, by comparison of the 16S nucleotide sequences, if the recently (Sokolova and Fuxa, 2001. *J. Eukaryot. Microbiol*, 48 Suppl:85S) discovered megaspores are the same microsporidian species and not a result of a mixed infection.

MATERIALS, METHODS AND RESULTS

Thelohania solenopsae is a microsporidian parasite of RIFA introduced into the Southern U.S. from South America in early 1930s. *T.solenopsae* produces at least five types of spores in providing for biological control of RIFA. It produces at least two types of spores known in the dipterygote spore, have been known since the description of the species by Knell and Allen in 1977 (*J. Invertebr. Pathol.* 29:192-200). Megaspores were described recently and an morphologically distinct from the two known spore types (Fig.1).

Fig.1 Light microscopy of three spore types discovered in the ant hemocoel. *A.Live spore in wet smears seed phase contrast: megaspore – red arrow, octospore - green arrow. Nosema-like spore - white arrow.* 40X obj. Bar - 10 um. *B. Spore stained with trichrome chromotrope-based stain: 1- individual octospore, 2 - octet of octospores, 3 - octet of octospores, containing two "macrospores" (octospores failed to divide), 4 - Nosema-like spore, 5 - spore stained with subculosar while H2R, spore types are indicative with C. Spore stained with fuchsin and DAPI for visualization of nuclei with DAPI only; stain nuclei of immature spore, 6 - penetrate poorly through the envelope of mature spore of all types. 100X obj. Bar - 5um D. Octospores stained with calcofluor and DAPI for visualization of nuclei with DAPI only; stain nuclei of immature spore, 6 - penetrate poorly through the envelope of mature spores of all types. UV filter A_ex=330-380 nm, magnification is the same as on fig C.*

Source and isolation of spores

Ants and spores used in the experiment were collected from test sites and were stained briefly. In laboratory, hemocoel smears from the infected ants were stained by Calcofluor, dense Nosema-like spores of the *T.s.* octet hemocoel. Laser-pressure catapulting of the *T.s.* spore types were picked up, individual octospores, while they were not individual, were confirmed as part of the *T.s.* octet hemocoel, while they are not individual, were confirmed as part of the *T.s.* octet hemocoel. The microdissection and megaspores were collected and computed by the LPC technique into a lid of a 0.5 ml reaction tube.

Fig.2 Smear before (A) and after (B,C) microdissection (arrows) from a selected microsporidian spores (as they were collected and computed onto the meniscus of PALM lid) Calcofluor, 40X obj.

DNA isolation and PCR

Polymerase chain reaction was performed on each spore type in the original montage tube. The initial PCR reaction mixture included a total volume of 20µl. 10 µl buffer H (Fabara PCR kit, Pushkin, Madison), WI. 1µl of each of 10 µM primers (see below), 0.9 µl Pallara enzyme mixture and the montage tube type. PCR was conducted in a MJ Research thermocycler 100 with a montage tube. Primers targeted amplified a spore types in place. The initial reaction mixture was added, the montage tube was added to the selected microsporidian primers V1F-630R of the *T.s.* DNA from spore types. However, a full clear sequence of their products has not obtained probably due to constant production of dimers (Fig-4b). The initial reaction mixture was placed into the cap of a reaction production of dimers (Fig-4b). The initial reaction mixture was heat treated and centrifuged. Once all components were added, the montage tube was subjected to PCR amplification with the universal microsporidian primers V1F/630R. The second PCR cycle parameters mirrored the same as in the first reaction.

Table.
Primers used for amplification and sequencing of 16S rDNA from *T. solenopsae*

Primer	Sequence 5' – 3'	Expected amplicon size	Obtained amplicon size*	Reference
1TS9		240 b	240 b	Vossbrinck et al, 2002 J. Invertebr. Pathol. 81:159-261
5TSAS				
18SV1F		330 b	160 b	Weiss, Solokova, Fuxa, unpubl.
TS18Ssr				

*The minimal obtained size from some sequences used for major

Agarose gel electrophoresis

PCR products were loaded on a 2% agarose gel, which was run at 150 KV and photographed with ethidium bromide. Electrophoretic patterns of fragments amplified by each of the primers used, were identical regardless of whatever the DNA was obtained from individual octospores, octets of octospores, megaspores, or from ants from different spore types. The number of octospores, megaspores, it did not affect the success of amplification (Fig. 4 A, B).

A. Fragments of 16S rDNA obtained from different numbers of *T.s.* octospores, and amplified with the TS9AS-F/TS18SGss primers. Lanes: 1, 10 - 100-base DNA size leader; 2 – 20 megaspores; 3 – 10 megaspores; 4 – 20 individual octospore; 5 – 10 individual octo-spore; 6 – 1 octet of octospores; 7 – 1 megaspore; 8 – negative control (no DNA); 9 - positive control (DNA isolated from the *T.s.*)

B. Comparison of 16S rDNA fragments obtained of *T.s.* individual octospores (lanes 2-4) or megaspores (lanes 5-7) amplified by each of three sets of primers: 1,9 – DNA size leader; 2,5 - TS9AS-F/TS18SGss fragments; 3,6 – V1F/630R (universal microsporidian primer); 4,7 – 1TS9-F/TS18Ssr; 8 - negative control.

Fig.3 Agarose gel electrophoresis of PCR products

Sequence analysis

The positive bands were extracted with a gel extraction kit (Qiagen, Omega, CA) to obtain pure DNA samples. The DNA samples were sequenced using direct forward primer. The resulting forward and reverse sequence data were assembled and sent to GenCide (School of Veterinary Medicine, Louisiana University) for sequencing. After direct sequencing of the amplicons, the resultant sequences were aligned with *T.s.* 16S rDNA sequence (AF134390) and compared between the two spore types. The resulting two 16S - base sequences corresponding to regions amplified by 1TS9 - 5TSAS and TS9AS-F/TS18SGss primer pairs of *T.s.* showed 100% homology between the two spore types and with the *T.s.* 16S rDNA sequence.

Fig.4 *Thelohania solenopsae* 16S rRNA gene, complete sequence. Acc AF134390 (Moser et al, 2000 *J. Invertebr. Pathol.* 75:174-177). Regions amplified for sequence analysis in octospores and megaspores of *T.s.* are shown in bold.

SUMMARY OF RESULTS

☑ A PCR protocol has been developed to achieve consistent amplification of 16S rDNA from eight spores obtained from smears;

☑ DNA obtained from 8 octospores or megaspores (or from one sporophorous vesicle containing 8 octospores) was sufficient for amplification and sequencing, and an increase in the spore number up to 20 did not affect the success of amplification;

☑ amplicon sizes and sequences of two fragments of *T. solenopsae* 16S rDNA were identical in octospores and megaspores, indicating they represent two distinct spore types belonging to one polymorphous species.

Laser Pressure Catapulting Microdissection techniques has been proved to be a valuable instrument that can be applied to research on microsporidia; it is the only existing instrument that allows genomic and proteomic analysis of infection in situations when the material for analysis is strictly limited.

Evaluation of Broadcast Applications of Cyfluthrin & Beta-Cyfluthrin Against Red Imported Fire Ants, *Solenopsis invicta*

Kelly Loftin[1], John Hopkins[1], Doug Petty[2], and, Donna Shanklin[3]

Introduction

The objective of this trial was to evaluate the efficacy of broadcast applications of 0.1% cyfluthrin granules, 0.05 % beta-cyfluthrin granules and a combination treatment of pyriproxyfen granular bait followed by 0.05% beta-cyfluthrin against red imported fire ants (RIFA), *Solenopsis invicta* (Figs 1, 2, 3). The trial consisted of 5 treatments replicated 3 times. The effectiveness of cyfluthrin, beta-cyfluthrin and the pyriproxyfen/beta-cyfluthrin combination was compared to that of granular fipronil. Evaluations were conducted by collecting foraging fire ants using hot dog baited traps (Jones et al. 1998) and by counting active mounds in each plot. The final evaluation included a determination of the presence of fire ant brood from RIFA colonies.

Materials and Methods

Study Site: The study was conducted in Texarkana (Miller Co.) located in southwest Arkansas. Density of red imported fire ant, *Solenopsis invicta* mounds at the study site was approximately 350 mounds per acre. Grass had been mowed approximately 3 days prior to the study.

Design: Each treatment and the untreated control were replicated three times. Plots were approximately 0.25 acre in size. The treatments and control were randomly allocated within each block. The three blocks corresponded to different areas of the study site.

Evaluation: Pre and post treatment evaluations were conducted using bait stations to collect foraging ants within each plot following the methods of Jones et al. (1998). Bait stations consisted of a 1/4 inch hot dog cube placed on a snap vial lid and marked with a wire survey flag. Ten bait stations were arranged as two transects with 5 bait stations each, located approximately 4.7 m (15 feet) on each side of the center line of each plot. Bait stations within each transect were approximately 4.7 m (15 feet) apart. Bait was made available to foraging ants for approximately 30 min. The number of RIFA collected from each station in each plot was determined to evaluate efficacy. Another method of evaluation was determining the number of active mounds at pre treatment and at post treatment intervals. Mound activity was determined by gently probing each mound with a 1/8 inch metal probe and estimating the number of ants responding in 20 seconds. Percentage of mounds with brood was determined during the final evaluation. Ten mounds in each plot were excavated using a spade and observed for the presence of fire ant brood (eggs, larva and pupa).

Insecticide Applications: Products were applied as specified in protocol supplied by Bayer. Products and application rates are given in Table 1. Insecticides were broadcast using a Herd® spreader mounted on a Kawasaki® ATV Mule equipped with a digital speedometer. After calibration, the specified rates were broadcast by matching to the appropriate ground speed and dispersal rate. Insecticide applications were made on July 1, 2002. Pyriproxyfen was applied to the combination pyriproxyfen/beta-cyfluthrin treated plots the same day all other treatments were made. Seven days later beta-cyfluthrin was applied to these plots and watered-in.

Statistical Analysis: Data collected from bait stations were analyzed using Analysis of Variance based on a RCB design with 3 replicates. The protected LSD procedure was used to determine significant differences in the mean number of foraging RIFA collected from the various treatments (Statistix 2000). The percentage of mounds containing brood was analyzed using the same procedures as above.

Table 1. Insecticide rates and manufacturer.

Insecticide	Rate	Manufacturer
0.1% cyfluthrin GR	191 lbs/acre	Supplied by Bayer
0.05% beta-cyfluthrin GR	191 lbs/acre	Supplied by Bayer
0.05% pyriproxyfen GR + 0.05% beta-cyfluthrin GR	10lb/acre + 191lb/acre	Spectracide Fire Ant Killer Plus Preventer
0.0103% fipronil GR	87 lb/acre	Supplied by Bayer Over N Out, GardenTech

Fig 1. Beta-cyfluthrin

Fig 2. Weighing pyriproxyfen bait

Fig 3. Fipronil granular

Table 2. Number of foraging RIFA following insecticide application

TRT	PRE	1DAT	2DAT	7DAT	14DAT*	30DAT	60DAT	90DAT	120 DAT	150 DAT	194 DAT
UTC	97.7a	55.9a	107.5a	112.3a	92.8a	83.8a	141.7a	69.0a	46.7a	40.3a	21.8a
Cyfluthrin	108.6a	39.7a	50.4a	54.6a	56.3ab	71.6a	74.5ab	44.5ab	30.2a	14.2a	6.4a
Beta-Cyfluthrin GR	80.9a	15.3a	57.0a	53.9a	56.6ab	69.3a	118.5a	22.4ab	8.2a	9.2a	6.8a
Pyriproxyfen & Beta-Cyfluthrin	73.7a	29.3a	44.0a	45.7a	37.2c	6.0a	7.9b	0.5b	2.3a	3.5a	6.8a
Fipronil GR	92.7a	34.7a	76.5a	73.1a	24.3c	7.2b	11.2b	0.1b	1.0a	3.3a	0.3a

Means followed by the same letter are not significantly different using the protected LSD mean separation procedure (α = 0.5) unless otherwise noted.
*For 14DAT, means followed by the same letter are not significantly different using the protected LSD mean separation Procedure (α = 0.10).

Table 3. Number of active RIFA colonies following insecticide application

TRT	PRE	1DAT	2DAT	7DAT	14DAT	30DAT	60DAT	90DAT	120 DAT	150 DAT	194 DAT
UTC	26.0a	23.7a	21.3a	19.3a	21.7a	12.7a	15.3a	11.0a	13.3a	23.0a	10.7a
Cyfluthrin	27.7a	27.0a	22.3a	22.7a	16.0a	7.3a	8.0abc	7.3a	11.0a	11.3ab	15.0a
Beta-Cyfluthrin GR	19.7a	13.3a	13.3a	13.7a	14.3a	5.0a	8.7ab	4.7ab	5.0a	5.3b	8.7a
Pyriproxyfen & Beta-Cyfluthrin	30.3a	23.0a	27.3a	24.0a	17.3a	7.0a	3.7bc	0.7b	1.3a	2.0b	2.3a
Fipronil GR	21.3a	19.0a	18.0a	16.3a	19.0a	5.7a	0.3c	0.7a	0.7a	1.0b	1.3a

Means followed by the same letter are not significantly different using the protected LSD mean separation procedure (α = 0.5)

Table 4. Percent reduction in active RIFA colonies, percent of active mounds with brood and overall brood reduction following insecticide application.

TRT	1DAT	2DAT	7DAT	14DAT	30DAT	60DAT	90DAT	120DAT	150DAT	194DAT	% with brood	%brood reduction
UTC	9b	20a	26a	16a	4c	48a	52a	35b	56b	93b	61a	
Cyfluthrin	2b	16a	18a	44a	75a	73a	63a	61a	49a	90a	57a	
Beta-Cyfluthrin	34a	37a	37a	62a	49bc	64a	67a	69a	54a	91a	53a	
Pyriproxyfen & Beta-Cyfluthrin	22ab	12a	20a	34a	74a	90a	97a	94a	94a	100a	89a	
Fipronil GR	17ab	20a	26a	9a	76a	97a	97a	92a	94a	50a	96a	

Means followed by the same letter are not significantly different using the protected LSD mean separation procedure (α = 0.5)

Table 5. Numerical ranking of RIFA control following insecticide treatments 1 = very good control, 9 = no control

TRT	1DAT	2DAT	7DAT	14DAT	30DAT	60DAT	90DAT	120 DAT	150 DAT	194 DAT
UTC	9 c	8.7a	7.3a	8.3a	4.7a	5.3a	5.0a	4.7a	6.0a	4.7a
Cyfluthrin	9 a	8.0a	8.3a	5.7a	2.3a	3.0ab	2.7a	4.0a	4.0a	5.0a
Beta-Cyfluthrin GR	6.3b	6.3a	6.3a	7.0a	4.0a	5.0a	3.7a	3.3a	3.7a	5.3a
Pyriproxyfen & beta-Cyfluthrin	8 ab	8.3a	8.0a	6.7a	2.7a	1.3b	1.0a	1.0a	1.0a	1.3a
Fipronil GR	6 ab	7.7a	7.3a	8.7a	2.7a	1.0a	1.0a	1.0a	1.3a	1.3a

Means followed by the same letter are not significantly different using the protected LSD mean separation procedure (α < 0.5)

Results and Discussion

The mean number of foraging fire ants collected from bait stations is given in Table 2. Although reductions in the number of foraging RIFA were noted 1 day after treatment (DAT), statistically significant reductions did not occur until 14 DAT. At 14 DAT both the pyriproxyfen/beta-cyfluthrin and fipronil treated plots had significantly fewer foraging RIFA than all other treatments and the untreated control. This trend held through 60 DAT. By 90 DAT, these treatments still had significantly fewer foraging RIFA than the untreated control. Lower numbers of foraging RIFA were found in the beta-cyfluthrin, pyriproxyfen/beta-cyfluthrin and fipronil treated plots from 120 through 194 DAT, however, this reduction was not statistically significant.

Results from active mound determinations were similar to results from foraging RIFA (Table 3). Reductions in the number of active RIFA mounds began occurring at 1 or 2 DAT for the insecticide treatments; however, this reduction was not statistically significant until 60 DAT. At 60 DAT significantly fewer active RIFA mounds were found in the pyriproxyfen/beta-cyfluthrin and fipronil treated plots when compared to the untreated controls. In addition, fipronil treated plots had significantly fewer active RIFA mounds when compared to the cyfluthrin and beta-cyfluthrin treated plots. This trend continued through 90 DAT. By 150 DAT however, statistical differences were only noted at 60 and 90 DAT.

Percent reduction in the number of active RIFA mounds is listed in Table 4. The greatest levels of reduction were noted with pyriproxyfen/beta-cyfluthrin and fipronil treated plots, however, statistical significance occurred only at 60 DAT. The percentage of active mounds containing brood at 194 DAT is presented in Table 4. This figure ranged from 50 to 93%. This figure can be deceiving because it is based only on the percentage of active mounds found in the plots at 194 DAT. For example, 100 % of the active mounds found in the fipronil treated plots contained brood however; an average of only 1.3 active mounds were found in these plots. A more accurate representation compares the number of mounds containing brood reduction at 194 DAT to pre-treatment mound numbers (Table 4).

Table 5 lists a numerical rating of control for all treatments. The rating is based on a 1-9 rating scale with a rating of 1 meaning excellent control and a rating of 9 meaning no control. Pyriproxyfen/beta-cyfluthrin and fipronil demonstrated the best level of control based on this rating scheme. High levels of control with these products began at 30 DAT and continued throughout 194 DAT. However, only the 60 DAT evaluation period demonstrated statistical significance (Table 5).

All measures of efficacy used in this trial suggest that both beta-cyfluthrin and the pyriproxyfen/beta-cyfluthrin combination would provide a level of control acceptable to homeowners. The results from previous trials evaluating pyriproxyfen bait (Loftin unpublished 1999) and granular fipronil confirm this finding (Loftin et al. 2003).

References

Jones, D., L. Thompson and K. Davis. 1998. Measuring Insecticide Efficacy: Counting Fire Ant Mounds vs. Bait Station Sampling. Proceedings of the 1998 Imported Fire Ant Conference. Hot Springs, Arkansas

Loftin, K., K. Hopkins, J. Gavin and D. Shanklin. 2003. Evaluation of broadcast applications of various insecticides against red imported fire ants, *Solenopsis invicta* Buren. Proceedings of the 2003 Red Imported Fire Ant Conference. Palm Springs, Florida. P. 77

Statistix 7. 2000. Analytical Software, Tallahassee, Florida

[1] Extension Entomologists, P.O. 391, Little Rock, AR 72203, kloftin@uaex.edu, jhopkins@uaex.edu
[2] Miller Co., CEA-Ag Ste 212, Texarkana, AR 71854, dpetty@uaex.edu
[3] Extension Entomologist, SEREC, P.O. Box 3508, Monticello, AR 71656, shanklin@uamont.edu

Evaluation of Various Cyfluthrin and Beta-Cyfluthrin Formulations as Individual Mound Treatments Against Red Imported Fire Ants

John D. Hopkins, Kelly Loftin, R. Allan Beuerman, and Donna Shanklin

University of Arkansas Cooperative Extension Service

Little Rock, Arkansas, USA

UNIVERSITY OF ARKANSAS
DIVISION OF AGRICULTURE
Cooperative Extension Service

Introduction

Solenopsis invicta Buren, the red imported fire ant (RIFA), arrived in Mobile, Alabama from South America between 1933 and 1945 (Callcott & Collins 1996). The first documented infestation in Arkansas occurred in 1958 (Loftin et al. 1999). Since 1958 it has spread through over 30 counties in the southern half of Arkansas. Economic losses including the cost of controls is estimated at nearly $42 million for Arkansas and $2.5 billion for the 11 RIFA infested states across the south (Thompson & Wiley 2002). Homeowners have many choices with respect to insecticides available for managing red imported fire ants around their homes. The objective of this trial was to evaluate the efficacy of a 1% cyfluthrin dust, a 0.1% cyfluthrin granule, a 0.5% beta-cyfluthrin dust, and a 0.5% beta-cyfluthrin granule as individual mound treatments against red imported fire ants, and compare their efficacy to commercial standards. This trial consisted of 9 treatments replicated 3 times. The effectiveness of permethrin, acephate, bifenthrin, and lambda-cyhalothrin were compared to that of the various rates and formulations of cyfluthrin and beta-cyfluthrin.

Materials and Methods

Study Site: Product evaluations were conducted in Little Rock, Arkansas (Pulaski Co.) Density of red imported fire ant, *Solenopsis invicta* mounds at the study site was approximately 230 mounds per acre indicating the presence of multiple queen (polygyne) colonies. Grass and other vegetation had been mowed approximately 1 week prior to the study.

Mound Selection: All visible mounds within the study area were flagged prior to initiation of the study. Flags were not placed directly into the mound but approximately two inches from the base of the mound (on the north side) to minimize disturbance. Treatment number, replicate number and mound number (ex. Trt 1, Rep 1, Number 1...Trt 1 Rep 1, Number 2...... Trt 1, Rep 1, Number 10) were permanently marked on each flag. Fire ant mounds receiving treatments were of similar size. Flags remained on all untreated mounds so that mound relocation could be determined.

Evaluation: Evaluation of fire ant populations was conducted 1 day pre-treatment then at 1 day after treatment (1 DAT), 2 DAT, 3 DAT, 7 DAT, 14 DAT, and 35 DAT. Fire ant activity was determined by gently probing each mound with a 1/8 inch metal probe. Then, the number of ants responding within 10 seconds were counted. The distance and activity of newly formed mounds found near treated mounds were recorded to determine percent mound relocation. Newly formed mounds located within 18 inches of a treated mound were considered to be a relocated mound. The presence of fire ants was determined during at final evaluation date by digging into the mound and searching for brood (eggs, larva or pupa).

Pesticide Application: Application rates for all treatments are listed in Table 1. Labeled methods and application rates for products currently labeled for fire ant control were used. Application methods for the experimental treatments were as listed below. One teaspoon of 1% cyfluthrin dust was sprinkled evenly over each of 10 mounds per each of three 1.0% cyfluthrin dust plots. One-quarter cup of 0.1% cyfluthrin granules was sprinkled evenly over each of 10 mounds per each of the granular cyfluthrin plots. The experimental beta-cyfluthrin 0.5% dust and 0.05% granular treatments were applied in the same manner as the cyfluthrin treatments at the rate of 1 teaspoon per mound and ¼ cup per mound, respectively. All other insecticide treatments were applied in a similar manner. Granular treatments were "watered in" by gently sprinkling 1 gallon of water over the treated mounds. Insecticide treatments were applied August 26, 2003.

Statistical Analysis: All Data were processed using Agriculture Research Manager Ver. 6.1.12. Analysis of variance was run and Least Significant Difference (P=0.05) was used to separate means only when AOV Treatment P(F) was significant at the 5% level.

Results and Discussion

No significant differences in the number ants responding to the probe were observed among treatments when rated 1 day pre-treatment. Significant ant reduction was noted at 1 DAT with all of the treatments except for the 0.25% permethrin dust (Table 2). Significant reduction in the number of RIFA per mound was not achieved with the permethrin treated mounds except at the 7 and 14 DAT evaluations. The greatest level of control throughout the study, based on ants responding to the probe, was achieved with acephate dust, bifenthrin granule, cyfluthrin granule, and beta-cyfluthrin granule and permethrin dust (Table 2).

Statistically, significant differences in mound relocation among treatments were not seen during the 35 day evaluation period (Table 3). Although not statistically significant one-third of the permethrin treated mounds relocated by 35 DAT followed by 26.7, 20.0, 13.3, 6.7 and 6.7% for cyfluthrin dust, beta-cyfluthrin dust, bifenthrin granular, lambda-cyhalothrin granular and the remaining treatments, respectively. Mound relocation was not noted for the remaining treatments.

The proportion of RIFA mounds containing brood at 35 DAT is given in Table 4. No RIFA mounds in the cyfluthrin granular and acephate dust treatments contained brood. A significantly lower proportion of RIFA mounds contained brood for the cyfluthrin granular, acephate dust, beta-cyfluthrin granular, lambda-cyhalothrin granular, and bifenthrin granular treatments. Estimated percent reduction in the proportion of RIFA mounds containing brood was 100,

100, 90 and 90 for the cyfluthrin granule, acephate dust, beta-cyfluthrin and lambda-cyhalothrin dust, respectively (Table 4). Percent control was calculated based on the numbers of ants responding to the probe at 1 day pre-treatment and at 35 DAT. Percent control for the treatments mirrored the results obtained for the number of ants responding to the probe.

Table 1. Products, rates, and manufacturer/lot numbers.

Treatment	Rate	Trade Name / Lot Number

Table 2. Efficacy of Beta-cyfluthrin and Various Commercial Standards as Mound Treatments for Red Imported Fire Ant Control. Pulaski Co., AR. 2003.
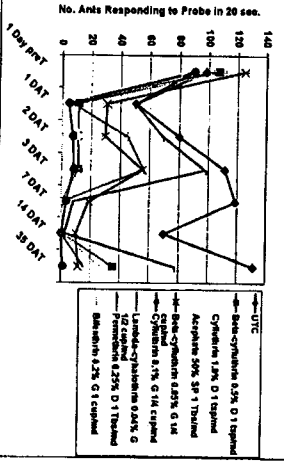
Table 3. Red Imported Fire Ant Mound Relocation Following Treatment With Beta-cyfluthrin and Various Commercial Standards. Pulaski Co., AR. 2003.

Table 4. Brood Presence and Percent Control of Red Imported Fire Ants Following Treatment With Beta-cyfluthrin and Various Commercial Standards. Pulaski Co., AR. 2003.

References

1. Callcott, A-M. A. & H. L. Collins. 1996. Invasion and range expansion of red imported fire ant (Hymenoptera: Formicidae) in North America from 1918-1995. Florida Entomol. 79: 240-251.

2. Loftin, K. M. D. Shanklin & D. Blackburn. 1999. The federal imported fire ant quarantine. University of Arkansas Cooperative Extension Service Fact Sheet FSA7053-2.5-9-99N. 4 pp.

3. Thompson, L. C. & S. Wiley. 2002. Annual losses caused by red imported fire ants to households in the southern U.S. pp. 109-115. In Proceeding, 2002 imported fire ant conference. Athens, Georgia. 175 pp.

KIDzANTS®: A New Curriculum about Fire Ants for Kids

N. Riggs, P. Nester & E. Brown
Texas Cooperative Extension

According to a 1998 study conducted by Dr. Curtis Lard et.al. from the Department of Agricultural Economics, TX A&M University, of red imported fire ant (Solenopsis invicta) related costs in Dallas, Fort Worth, Austin, San Antonio, and Houston, red imported fire ants have serious economic effects for these metro areas of Texas. Households experienced the largest costs among sectors examined with a average of $151 per households spent annually which included repairs to property and equipment, first-aid. pesticides, baits, and professional services. A full damage assessment for Texas must include additional sectors, and the estimated costs of $581 million per year for the selected sectors underscore the impact of this pest. Treatment costs accounted for over 50% of this total cost. In Houston the average medical treatment costs per household of $25.46. The duration of injury for children and adults was 6.6 days and 5.6 days, respectively. Education of the general public about health and safety issues concerning fire ants is important. Understanding fire ant biology is essential before the public can understand the specifics of how baiting products work, and the concepts that make up the baiting program that the Texas Cooperative Extension recommends for the control of the fire ant. Innovative methods for the presentation of concepts are always needed so teachers/volunteers can accurately present information on fire ants in a method that is both appealing to the respective audience and satisfying to the teacher/volunteer. Focusing on elementary children can be an excellent avenue for getting a message concerning fire ants and fire ant safety home to parents. The KIDzANTS curriculum with educational CD and website **(http://kidzants.tamu.edu)**was developed, containing six 'learning experiences' to educate young children about the fire ant. This curriculum includes 6 lessons covering the introduction of the fire ant to the United States, morphology, life cycle (queen, workers, brood, and mating flight), mound development (single vs. multiple queen and structure), identification versus other ant species, impact on wildlife, health and safety issues, and the diet of the fire ant. Activities requiring total class involvement have been included. Students in 3rd, 4th and 5th grade science classes are targeted.
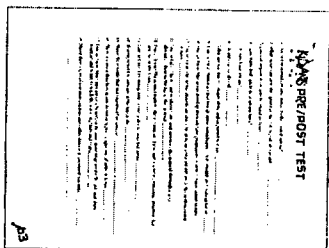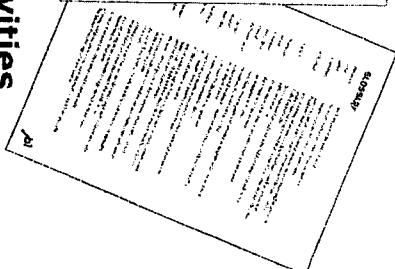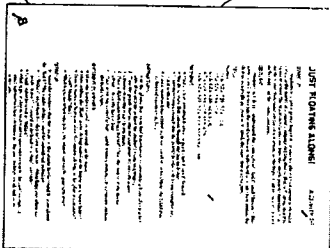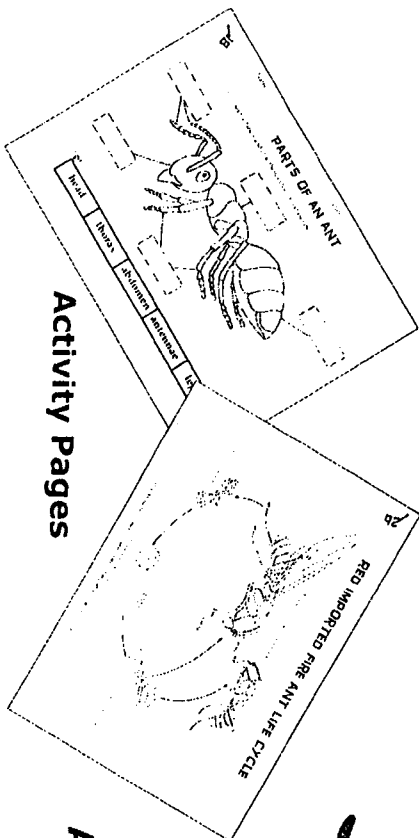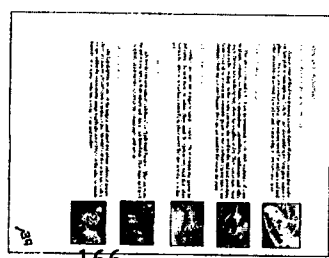
Leader Lesson Guide

Several Lessons Various Topics

Lessons and Activities

Testing

Group Participation

Activity Pages

A New Curriculum About Fire Ants for Kids

Posters and Games

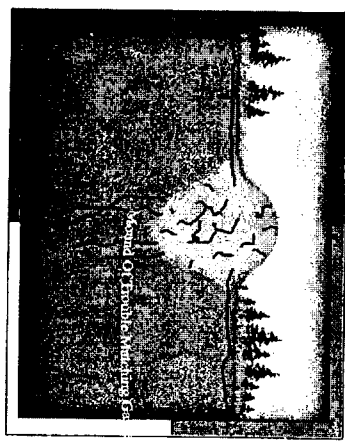PARTS OF AN ANT

head | thorax | abdomen | antennae | leg

RED IMPORTED FIRE ANT LIFE CYCLE

According to a 1998 study conducted by Dr. Curtis Lard et.al. from the Department of Agricultural Economics, TX A&M University, of red imported fire ant (Solenopsis invicta) related costs in Dallas, Fort Worth, Austin, San Antonio, and Houston, red imported fire ants have serious economic effects for these metro areas of Texas. Households experienced the largest costs among sectors examined with a average of $151 per households spent annually which included repairs to property and equipment, first-aid, pesticides, baits, and professional services. A full damage assessment for Texas must include additional sectors, and the estimated costs of $581 million per year for the selected sectors underscore the impact of this pest. Treatment costs accounted for over 50% of this total cost. In Houston the average medical treatment costs per household of $25.46. The duration of injury for children and adults was 6.6 days and 5.6 days, respectively. Education on the general public about health and safety issues concerning fire ants is important. Understanding fire ant biology is essential before the public can understand the specifics of how baiting products work, and the concepts that make up the baiting program that the Texas Cooperative Extension recommends for the control of the fire ant. Innovative methods for the presentation of concepts are always needed so teachers/volunteers can accurately present information on fire ants in a method that is both appealing to the respective audience and satisfying to the teacher/volunteer. Focusing on elementary children can be an excellent avenue for getting a message concerning fire ants and fire ant safety home to parents. The KIDzANTS curriculum with educational CD and website (http://kidzants.tamu.edu) was developed, containing six 'learning experiences' to educate young children about the fire ant. This curriculum includes 6 lessons covering the introduction of the fire ant to the United States, morphology, life cycle (queen, workers, brood, and mating flight), mound development (single vs multiple queen and structure), identification versus other ant species, impact on wildlife, health and safety issues, and the diet of the fire ant. Activities requiring total class involvement have been included. Students in 3rd, 4th, and 5th grade science classes are targeted.
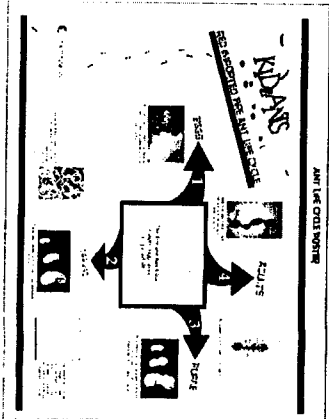
CONTENTS

INVADERS BY SEA

JUST FIGHTING ALONG!

KIDzANTS PRE/POST TEST

ANT LIFE CYCLE POSTER

EXTENSION

Texas Cooperative

Visit us at http://kidzants.tamu.edu

- 166 -

Interstate collaborative efforts to develop education programs for fire ant management in cattle production systems.

Kathy L. Flanders, Auburn University and Bastiaan M. Drees, Texas A&M University.

In the past year, the authors have worked together on developing educational programs on fire ant management in cattle operations. Programs included a workshop, an instructional DVD. a streaming video archive on the Internet, and a printed publication.

The workshop was held on April 15,2003. County agents and cattlemen at three sites in Alabama were connected to each other via Internet videoconferencing. The training session for Alabama stakeholders was conducted by Drs. Bart Drees and Charles Barr from College Station, Texas. We had planned for the instructors to be connected with the Alabama groups via videoconference. However, a power outage in College Station forced the instructors to narrate their PowerPoint Presentations via cell phone, while the actual presentations were broadcast from Auburn, Alabama. Despite the technical difficulties, the format allowed ample opportunity for interaction between the experts in Texas and the stakeholders in Alabama. Drs. Drees and Barr went to the studio of the Communications Department of Texas Cooperative Extension, and made the presentations again, so that they could be recorded. The result is a DVD containing two presentations, "Managing Fire Ants in Cattle Operations," and "Managing Fire Ants in Agriculture." The DVD was reproduced for distribution in Texas and in Alabama. In order to make the information more accessible, the communications department of the Alabama Cooperative Extension System archived the two videos as streaming video and posted them on the Internet. Copies of the DVD will be made available on request, or you can view the presentations at the following links:

Managing Fire Ants in Agriculture
http://www.aces.edu/extcomm/satellite/agriculture.wmv
Managing Fire Ants in Cattle Operations
http://www.aces.edu/extcomm/satellite/cattle.wmv

A stand alone publication on managing fire ants in cattle operations was produced, and is now available as a joint publication of the Alabama Cooperative Extension System and Texas Cooperative Extension (Circular ANR-1248, Managing Fire Ants in Cattle Production Systems).

These educational programs are the latest collaborative efforts between the Alabama Cooperative Extension System and Texas Cooperative Extension. A fire ant video, Fire Ant Control Made Easy, was produced in Alabama, revoiced in Texas for distribution there, translated into Spanish in Texas, and has now come full circle, back to Alabama as archived streaming video:

Control Facil de las Hormigas Bravas
http://www.aces.edu/extcomm/satellite/ez_esp.wmv

Other fire ant educational materials are available at:

Texas Imported Fire Ant Applied Research and Education Program web site:
fireant/tamu.edu
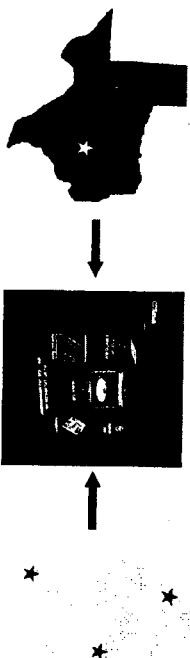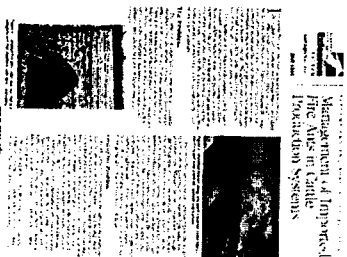Alabama Cooperative Extension System web site for fire ants:
www.aces.edu/dept/fireants

Interstate collaborative efforts to develop education programs for fire ant management in cattle production systems.

Kathy L. Flanders, Auburn University and Bastiaan "Bart" M. Drees, Texas A&M University.

In the past year, the authors have worked together on developing education programs on fire ant management in cattle operations. Programs included a workshop, an instructional DVD, a streaming video archive on the Internet, and a printed publication.

Internet Videoconference, April 2003

The workshop was held on April 15, 2003. County agents and cattlemen at three sites in Alabama were connected to each other via Internet videoconferencing. The training session for Alabama stakeholders was conducted by Drs. Bart Drees and Charles Barr from College Station, Texas. We had planned for the instructors to be connected with the Alabama groups via videoconference. However, a power outage in College Station forced the instructors to narrate their PowerPoint presentations via cell phone, while the actual presentations were broadcast from Auburn, Alabama. Despite the technical difficulties, the format allowed ample opportunity for interaction between the experts in Texas and the stakeholders in Alabama.

DVD

Drs. Drees and Barr went to the studio of the Communications Department of Texas Cooperative Extension, and made the presentations again, so that they could be recorded. The result is a DVD containing two presentations, "Managing Fire Ants in Cattle Operations," and "Managing Fire Ants in Agriculture." The DVD was reproduced for distribution in Texas and in Alabama.

Color Fact Sheet

A stand alone publication on managing fire ants in cattle operations was produced, and is now available as a joint publication of the Alabama Cooperative Extension System and Texas Cooperative Extension (Circular ANR-1248, Managing Fire Ants in Cattle Production Systems).

Streaming Video

In order to make the information more accessible, the Communications Department of the Alabama Cooperative Extension System archived the presentations on the DVD as streaming video and posted them on the Internet. Copies of the DVD will be made available on request, or you can view the presentations at the following links:
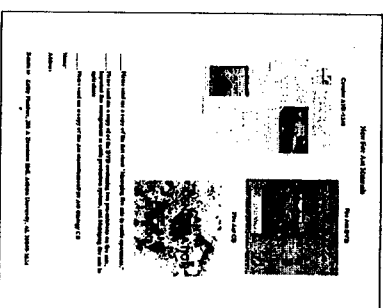
Managing Fire Ants in Agriculture
http://www.aces.edu/extcomm/satellite/agriculture.wmv

Managing Fire Ants in Cattle Operations
http://www.aces.edu/extcomm/satellite/cattle.wmv

Other Collaborative Efforts

These educational programs are the latest collaborative efforts between the Alabama Cooperative Extension System and Texas Cooperative Extension. A fire ant video, Fire Ant Control Made Easy, was produced in Alabama, revoiced in Texas for distribution there, translated into Spanish in Texas, and has now come full circle, back to Alabama as archived streaming video:

Control Fácil de las Hormigas Bravas
http://www.aces.edu/extcomm/satellite/ez_esp.wmv

Acknowledgments

RIFA, Solenopsis invicta Management in a South Louisiana Citrus Orchard

Dale Pollet, Patricia Beckley, Boris Castro[1], and Bobbie Fletcher[2]
[1]Department of Entomology, Extension, Louisiana State University
Life Sciences Building, Baton Rouge, Louisiana 70803
[2]Area Agent, Horticulture, Cooperative Extension Service,
Thibodaux, Louisiana, Lafourche Parish

ABSTRACT

The area wide community/subdivision program has been a very successful program in managing the red imported fire ant, Solenopsis *invicta.* The adoption of such a program to an agricultural system was evaluated. A demonstration was established in a citrus orchard that was used in an orchard verification program. The insect growth regulator, Extinguish, active ingredient, methoprene, was used as the test material due to the broad label use pattern. Applications were made in May and October 2003 using a Herd Seed Spreader adapted for RIFA bait application mounted to a 4-wheeler. The 15-acre orchard was divided into *6* blocks, 2 treatments of 1 lb/acre, 2 treatments of 1.5 lbs/acre and 2 controls. Mound counts and trapping of foraging ants was done monthly. Heavy rains and some flooding of the low end of the orchard affected initial foraging response for the ants. Extinguish effectively reduced RIFA populations as detected by reduced mound and foraging ant counts. The test was shortened due to conflict between the land owner and leaser preventing application in April 2004. We were permitted by the land owner to sample populations through May 2004 at which time the demonstration was terminated.

RED IMPORTED FIRE ANT MANAGEMENT IN SOUTH LOUISIANA CITRUS ORCHARD

Dr. Dale Pollet, Patricia Beckley, Dr. Boris Castro and Bobbie Fletcher

Introduction

Area wide management has worked effectively in communities and subdivisions. Application of the management system was demonstrated in a citrus orchard in South Louisiana. RIFA management in agricultural systems would enable farmers to reduce both direct and indirect costs.

Objective

The purpose of our demonstration, was to test the long term effect of a bait treatment on the established population of red imported fire ants in a Louisiana Citrus orchard. Baits have been a successful management tool in other settings, such as area wide programs (communities/subdivisions) but not in commercial citrus orchards in Louisiana.

Methods

The citrus orchard selected in LaRose, Louisiana was 15 acres and produced primarily Satsuma. The Orchard was divided up into four treatment blocks (average size 2.2 Acres each and two untreated control plots (average size 2.7 acres each)

Active fire ant mounds were counted and recorded by block. This was done each month to monitor active colony growth. Deep glass vials containing a ½ inch cube of Vienna sausage were placed evenly within each block. A total of 60 vials were placed under the trees throughout the treated and untreated plots. The vials were picked up after 1 hour. The number of ants in each vial were identified & counted in order to monitor foraging activity for one year.

Extinguish Fire ant bait was applied to the 4 blocks using a 4 wheeler and spreader mounted on the back. The bait was applied at 1 ½ lbs per acre in block A & C, and applied at 1 lb/A in blocks B & D.

The first bait treatment and first active mound and foraging ant count began on May 28, 2002. The second bait treatment was applied in the Fall, October 2, 2002.

Results

The density of the RIFA population was reduced by the bait treatments. Both the 1 lb and 1.5 lb rate worked effectively. Counts between the two rates of bait fluctuated due to heavy rain fall. The plots with the 1.5 lb rate started out with higher mound counts, then along with poor drainage kept the area wet. The end result was less problems with ants in the orchard equipment, less stings to the orchard workers and less exposure to pesticides due to the application of Extinguish growth regulator bait.
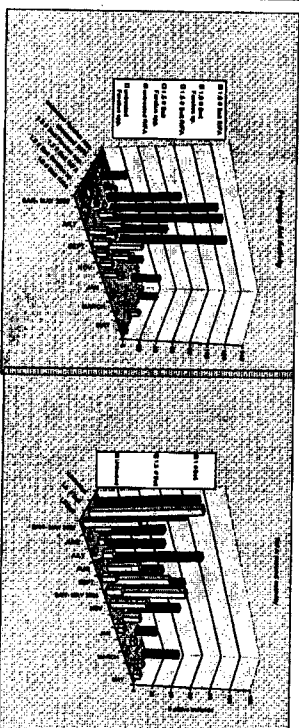
The other species of ant trapped in the vials was identified as *Forelius spp.* They are considered to be a competitor species.

Summary

Extinguish treatments at both rates lowered RIFA populations. Due to this success, several growers began using this program to manage RIFA populations in orchards. The third application was not made due to limited access to the orchard and problems with owner/leaser.

Untreated control 1		Untreated control 2
Block D 1 lb Bait/A		Block B 1 lb Bait/A
Block C 1 ½ lbs Bait/A		Block A 1 ½ lbs Bait/A
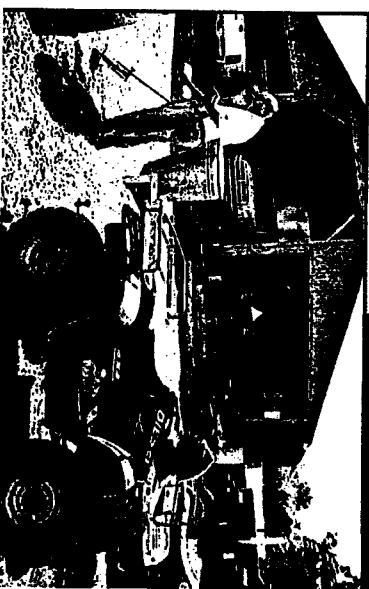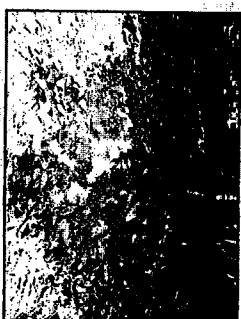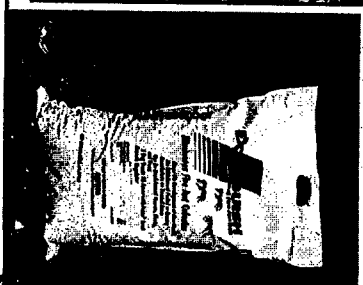
ABSTRACT

Initiation of Flight-Muscle Histolysis in
Red Imported Fire Ant *Solenopsis Invicta*

T. Azizi and S.B. Vinson
Department of Entomology Texas A&M University

The role of mating, flight, CO_2 level, juvenile hormone (JH), 20-Hydroxyecdyson and male lipoproteins transferred to the queen during mating in flight muscle histolysis of adult virgin fire ant queens were investigated. At the biochemical level, two novel proteins (A&B) were identified in the hemolymph of newly collected mated queens and were not present in the hemolymph of either virgin queens or in queens two days post-mating. In a reverse genetic approach we were able to find a 252 bp DNA Fragment corresponding to protein B that is homologous to CD27 proapoptotic mus musculus (siva) protein and cysteine protease inhibitor (CPI) in plants. Similarly a 671 bp PCR product was generated corresponding to protein A that showed homology to Lactate Dehydrogenase (LDH). Based on our findings, we are suggesting that these two proteins (putative fire ant queen CPI or SIVA and LDH) may be involved in flight muscle histolysis. The expression of putative siva or CPI and LDH were monitored at transcriptional levels to establish their role in flight muscle histolysis. A significant increase in the expression of putative siva or CPI in a mated queen was observed as compared to an alate virgin queen. The enhancements in the expression of putative siva or CPI started from day 0 post-mating and continued to increase for another 6 days until on day 7 the expression almost vanished. A rapid induction in the expression of putative LDH was also observed as revealed by northern blot analysis in the conditions essentially described earlier. On our second experimental approach, we found that topical application of methoprene (JH analog) at concentrations of 0.44 ng per ant stimulated 90% of alates to shed their wings. Artificial insemination also resulted in apoptotic nuclei in flight muscles. In contrast, other factors did not induce flight muscle histolysis in fire ant queens nor did they cause any significant dealation.

Authors: Nannan Liu, and Lee Zhang

Title: Gene Overexpression Associated with Workers of the Red Imported Fire Ant, *Solenopsis invicta* Buren

Abstract: Two cytochrome P450 genes, *CYP4AB1* and *CYP4AB2,* and the *Gp-9* gene were identified as being specifically overexpressed in workers of the red imported fire ant. The cDNA sequences of *CYP4AB1* and *CYP4AB2* have open reading frames of 1389 and 1533 nucleotides encoding proteins of 463 and 511 amino acid residues, respectively. Northern blot analysis was performed to compare expression levels of *CYP4AB1*, *CYP4AB2*, and *Gp-9* for different developmental stages and castes of fire ants. We demonstrate that the expression of these three genes is developmentally and caste specifically regulated in red imported fire ants. Levels of *CYP4AB1* mRNA were undetectable in $3^{rd}+4^{th}$ instars, worker pupae, and alate (mixed sex) pupae; readily detectable in male and female alates; increased in the queens; and rose to a maximum in workers. Similarly, the expression of *CYP4AB2* mRNA was undetectable in $3^{rd}+4^{th}$ instars, worker pupae, and alate pupae; low in male and female alates and queens; and increased in workers. Levels of *Gp-9* mRNA were readily detectable in male alates; increased in female alates; and reached a maximum in workers. Their caste-specific overexpression suggests the functional importance of *CYP4AB1*, *CYP4AB2*, and Gp-9 in workers of the red imported fire ant.

Gene Overexpression Associated with Workers of the Red Imported Fire Ant,
Solenopsis invicta Buren

Nannan Liu and Lee Zhang

Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849-5413 USA

Abstract

Two cytochrome P450 genes, CYP4AB1 and CYP4AB2, and the Gp-9 gene were identified as being specifically overexpressed in workers of the red imported fire ant. The cDNA sequences of CYP4AB1 and CYP4AB2 have open reading frames of 1389 and 1533 nucleotides encoding proteins of 463 and 511 amino acid residues, respectively. Northern blot analysis was performed to compare expression levels of CYP4AB1, CYP4AB2, and Gp-9 for different developmental stages and castes of fire ants. We demonstrate that the expression of these three genes is developmentally and caste specifically regulated in red imported fire ants. Levels of CYP4AB1 mRNA were undetectable in 3rd+4th instars, worker pupae, and alate (mixed sex) pupae; readily detectable in male and female alates; increased in the queens; and rose to a maximum in workers. Similarly, the expression of CYP4AB2 mRNA was undetectable in 3rd+4th instars, worker pupae, and alate pupae; low in male and female alates and queens; and increased in workers. Levels of Gp-9 mRNA were readily detectable in male alates; increased in female alates; and reached a maximum in workers. Their caste specific overexpression suggests the functional importance of CYP4AB1, CYP4AB2, and Gp-9 to workers of the red imported fire ant.

Introduction

Differential gene expression rather than genetic polymorphism has resulted in polyphenism, or alternative morphology, in many insect species (1) and underlies a range of biological and physiological processes, including development, reproduction, and behavior (2). Queens and workers of the red imported fire ant, *Solenopsis invicta* Buren, are alternative forms of adult females and display a good instance of insect polyphenism. These alternative forms with different physiological functions enable fire ants to use their amazing evolutionary success, harmonize each other and maintaining the complexity and order of social insect life. The substantial physiological differences reflect the differential expression of genes present these castes. Changes in gene expression that are associated with the evolution of wingless worker caste have been described in detail (3). In the current study, with an attempting to identify genes differentially expressed between queens and workers of the red imported fire ant, we identified first two cytochrome P450 genes, CYP4AB1 and CYP4AB2, and Gp-9 gene specifically overexpressed in workers of the red imported fire ant and has recently been discovered (4). Different alleles of Gp-9 in the workers enable them to recognize queens and regulate their numbers in the colony.

Materials and Methods

RNA isolation and cDNA preparation. Total RNA from fire ants was extracted using the acidic guanidine thiocyanate and phenol-chloroform method (5). Poly(A)RNA was isolated with Oligotex-dT suspension (QIAGEN). Double-stranded cDNAs were synthesized from poly(A) RNAs using the PCR-selected cDNA Subtraction Kit (Clontech). The double-stranded cDNAs were digested with RsaI to create smaller blunt-ended fragments to be used as testers (i.e. from workers) or drivers (i.e. from queens) according to the manufacturer's instructions (Clontech).

Subtractive cDNA library preparation. Two-step subtractive hybridizations were performed according to the manufacturer's instruction. The subtractive worker-queen (tester versus driver) library was constructed by directly cloning PCR-cDNA products that were recovered after subtraction into PCR 2.1 vector with a PCR 2.1 Original TA Cloning Kit (Invitrogen).

cDNA array. Plasmid DNAs were isolated from randomly selected cDNA clones of the worker-queen subtractive library by the alkaline-lysis method. The plasmid DNAs were arrayed onto parallel Nytran membranes and hybridized with 32P labeled cDNA probes of worker and queen synthesized by SuperScript II reverse transcriptase (Gibco BRL).

Cloning and sequencing of CYP4AB1 and CYP4AB2 3' and 5'-RACE was carried out using the Marathon cDNA Amplification Kit (Clontech) as described by the manufacturer. The double-stranded cDNAs were amplified by PCR. The PCR products (double-stranded cDNA fragments) were cloned into PCR 2.1 Original TA cloning vector (Invitrogen) and sequenced by automated sequencing (Genomic & Sequencing Lab, Auburn University).

Northern blotting analysis. Northern blot analyses were performed according to Sambrook et al (5). The cDNA fragments of differentially expressed candidate clones were labeled with [α-32P] dCTP and hybridized with RNA blots. The amount of RNA loaded in each lane was standardized by comparing the density of the 18S ribosomal RNA (rRNA) band on the agarose gel under UV light.

Results and Discussion

cDNA array and northern blot analysis. We initially screened a total of 400 clones from the worker-queen subtractive library with 32P labeled cDNA probes of whole bodies of workers and queens. Fifteen differentially expressed candidate clones from the library were identified and the cDNAs derived from these putative differentially expressed clones were used as probes for Northern analyses to further confirm differentially expressed patterns. Northern blotting results indicated that 3 of the 15 clones showed consistent differences in their expression between fire ant workers and queens (Fig. 1).

Figure 1. Northern blot analysis of differentially expressed patterns from the cDNA arrays. mRNAs were isolated from the whole bodies of fire ants.

Clones #25 and #138 exhibited significant protein homologies with cytochrome P450 family 4, and clone #101 showed 100% amino acid sequence homology with a pheromone binding protein, Gp-9 (6). Clones #92 and #225 had no database match to proteins in the GenBank.

Cloning and sequencing of putative cytochrome P450 genes. The cDNA sequences of full-length clones #138 and #25 have open reading frames of 1389 and 1533 nt encoding putative protein of 463 and 511 amino acids, respectively. The putative protein sequences of clones #138 and #25 shared 43 to 48% identity with both *Blaberus discoidalis* CYP4C1 and *Helicoverpa zea* CYP4M7, and were thus named CYP4AB1 and CYP4AB2, respectively. The putative protein CYP4AB1 and CYP4AB2 shared 71% identity with each other (Fig. 2).

Fig. 2. Alignment of the cDNA/deduced amino acid sequences of CYP4AB1 and CYP4AB2. Conserved amino acid residues between CYP4AB1 and CYP4AB2 are indicated in red. Invariant and highly conserved motifs in the P450 proteins are underlined.

The P450 protein signature motif, FXXGXRXCXG, is present at amino acid residues 398-407 and 446-456 of CYP4AB1 and CYP4AB2, respectively, and the cysteine residue in this motif is known to be an important ligand for heme binding. An important characteristic of family 4, but not other P450s, is a conserved 13-residue sequence, EVVTFMFRGHDTT. This motif is present in its entirety at amino acid residues 360-372 of CYP4AB1 and 308-320 of CYP4AB2.

Expression analysis of CYP4AB1, CYP4AB2, and Gp-9. The molecular basis underlying insect polyphenism is differential gene expression (1). It was thus of interest to determine the expression profile of CYP4AB1, CYP4AB2, and Gp-9 mRNAs between different castes and among different developmental stages of fire ants. Northern blot analysis was performed to compare expression levels of these three genes for different developmental stages (3rd+4th instar larvae, pupae, and adult) and castes of fire ants. Levels of CYP4AB1 mRNA were undetectable in 3rd+4th instars and worker and alate pupae; were readily detectable in male and female alates; increased (~3-fold) in queens, and rose to a maximum (about 13-fold) in workers (Fig. 3A, B). Similarly, the expression of CYP4AB2 mRNA was undetectable in 3rd+4th instars and alate pupae, low in male and female alates and queens, and increased in workers (Fig 3A, C).
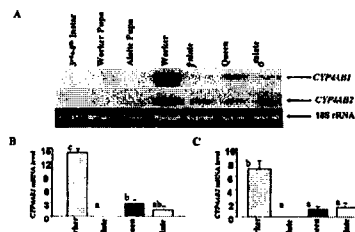

Figure 3. Expression analyses of CYP4AB1 and CYP4AB2 for different life stages and castes of red imported fire ants. The ethidium bromide stain of 18S ribosomal RNA in agarose gel is shown at the bottom. A: Northern blot analysis of CYP4AB1 and CYP4AB2 for different life stages of fire ants. B: Relative CYP4AB1 mRNA levels. C: Relative CYP4AB2 mRNA levels.

Levels of Gp-9 mRNA were readily detectable in male alates, increased (~3-fold) in female alates, and reached a maximum (~13-fold) in workers (Fig. 4A, B).
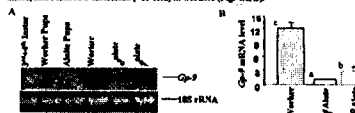

Figure 4. Expression analysis of Gp-9 for different life stages and castes of red imported fire ants. A: Northern blot analysis of Gp-9 for different life stages and castes of fire ants. B: Relative Gp-9 mRNA levels for different life stages and castes of fire ants.

The caste-specific expression of CYP4AB1, CYP4AB2, and Gp-9 suggests the functional importance of these genes in the worker caste of the red imported fire ant.

Summary

CYP4AB1, CYP4AB2, and Gp-9 gene were overexpressed in workers of the red imported fire ant. Northern blot analysis demonstrated that the expression of these three genes was developmentally regulated and caste-specifically expressed in workers, which suggests the functional importance of CYP4AB1, CYP4AB2, and Gp-9 in the workers of fire ants. However, there have been no previous reports of P450s characterized with respect to biological functions in the social insects, including the red imported fire ant. The availability of full-length sequences of CYP4AB1 and CYP4AB2 has made investigation of the function of P450 genes in fire ants possible. Different alleles of the Gp-9 gene in fire ants and workers play important roles in recognition of queens and regulation of their numbers in the colony. Investigation of the function of the gene overexpressed in the worker will extend our understanding of how a single gene functions as part of the social behavior of insects.

References

1. Evans, J.D., Wheeler, D.E. 2001. BioEssays 23, 62-68.
2. Nijhout, L.G., Jones, A.A., 1998, Annu. Rev. Entomol. 43, 671-700
3. Abouheif, E.; Wray, G.A., 2002. Science 297, 249-252.
4. Krieger, M.J.B. and Ross, K.G., 2002. Science 295, 328-332.
5. Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. In: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 7.37-7.50.

Appendix A

Aggression, Primer Pheromone, and Biogenic Arnines

R.K. Vander Meer[1], C.A. Preston[1], and A. Hefetz[2]

[1] USDA/ARS – CMAVE, Gainesville, FL 32608

[2] University of Tel Aviv, Department of Zoology, Tel Aviv, Israel

Nestmate recognition is a crucial element in the social organization of ants, operating as the first line of defense in maintaining colony integrity by excluding intruders. Adaptations of an animal's behavior to environmental and developmental change result from functional modifications in their central nervous system, which in turn is modulated by the release of various neurotransmitters and neurohormones. Octopamine (OA) and tyramine (Tyr) arc considered the functional equivalents of norepinephrine and epinephrine in invertebrates. In insects studied to date, OA acts as a neurohorrnone, neurotransmitter, and/or a neuromodulator. Accumulating evidence suggests that OA increases pheromone acuity in insects by lowering the threshold of response and increasing tlie sensitivity of pheromone specific neurons, but not neurons for general odors. We are working to unravel the role of OA and other biogenic amines in the social bcliavior of ants, specifically processes related to nestmate recognition in the red imported fire ant. We determined the roles of the queen and workers in nestmate recognition and the modulatory effects of biogenic amines on this process by manipulating biogenic arnine levels in workers. Ten queenright colonies were separated into the following three sub units: **A)** queenright (QR) ‐ fed crickets and 20% aqueous sucrose solution; B) queenless (QL) ‐ fed crickets and 20% aqueous sucrose solution; and C) queenless (QL) – fed crickets and an aqueous solution containing 20% sucrose and 1%

OA. Aggression bioassays were carried out weekly. The level of OA in worker brains was analyzed (t = 9-10 weeks). The level of OA decreases significantly in the absence of the colony queen. QL ants experience a significant reduction in OA levels that is redeemed by feeding the ant with OA. QR colonies maintain aggression levels between 5 and 7 while QL workers fed only crickets and aqueous sucrose had mean aggression levels between 2 and 4. Workers fed OA initially showed decreased aggression levels, as expected for QL worker groups, but aggression levels increased until they were not significantly different from the QR. Feeding OA to fire ant workers was adequate to simulate the presence of the queen, in terms of nestmate recognition, and presumably the release of the recognition primer pheromone. Thus, we have strong evidence that the queen recognition primer pheromone acts on workers to maintain high levels of OA that up - modulates worker sensitivity to the subtle changes in intraspecific nestmate recognition cues. This work represents a major step in unraveling the physiological effects of an ant primer pheromone.

The *Solenopsis* invicta Alarm Pheromone

C.A. Preston and R.K. Vander Meer

USDA/ARS – CMAVE, Gainesville, FL 32608

As with most social insects, the red imported fire ant, Solenopsis invicta, utilizes a complex milieu of chemical signals to regulate the activities of the colony. Several of these pheromones, including the trail pheromone and queen recognition pheromones, have been identified. However, the identification of the alarm pheromone has proven to be more elusive. Generally, alarm pheromones are associated with the mandibular gland, but they have also been identified in the Dufour's and anal glands. Behavioral studies point to the mandibular gland as the source S. invicta's alarm pheromone. Several functions of the alarm pheromone have been suggested for *S.* invicta, from signaling the presence of a threat, to inducing worker activity during mating flights, as well as attracting eavesdropping parasitoids. Wilson demonstrated that frenzied behavior was elicited by exposing *S.* invicta workers to volatiles released from crushed heads of conspecifics. Similarly, workers responded with frantic, excited movements when exposed to live alates, crushed heads, and mandibular gland solutions. The morphology of the gland and the ephemeral nature of the components have complicated the identification of this pheromone. In S. invicta, the mandibular gland consists of only a few cells, making it difficult to successfully isolate without the loss of materials. The chemistry of ant alarm pheromones is incredibly diverse with identified alarm

pheromones spanning many structural families, including terpenoids, alcohols, aldehydes, ketones, esters, and nitrogen heterocycles. The compounds are highly volatile, a necessity of quick but transient information transfer. We employed two analytical techniques, solid phase microextraction (SPME) and purge and trap, to collect and analyze headspace contents about workers exhibiting alarmed behavior. These techniques also eliminate the need for solvent, which complicates analysis of highly volatile compounds using the gas chromatograph-mass spectrometer (GC-MS). Using these techniques, we identified a substituted pyrazine as a component of the fire ant alarm pheromone. We tested this chemical in a behavioral bioassay with *S. invicta* workers and we found no significant difference between the alarm response to the headspace volatiles collected above shaken ants and the pyrazine at a concentration of 100 ng/μL, supplying a headspace concentration of 0.50 pg/μL. Additionally, compared to the response to headspace volatiles collected above unshaken ants, we found a significant alarm response to concentrations as low as 1 ng/μL, with a corresponding headspace concentration below detection limits (less than 0.03 pg/μL). We are continuing to define the alarm pheromone source and chemistry.

Fire Ant Repellents: Protection of Black-capped Vireos from Fire Ant Predation

R.K. Vander Meer[1], C.A. Preston[1], D. Cataldo[2], J.D. Cornelius[3], and C. Pekins[3]

1 USDA/ARS – CMAVE, Gainesville, FL 32608.
2 BioGuard R&D, Inc., Richland, WA 99352
3. DoA, Endangered Species Management, Ft. Hood, TX 76544

We have discovered and patented several chemical classes of repellent that have the potential to exclude fire ants from areas where they are not wanted. Examples of areas of use are electrical switch boxes, environmentally sensitive areas where insecticides are not permitted or reduction is mandated, e.g. State and National parks and military bases. The repellents are volatile and for long-term use require sustained release formulations. We have partnered with BioGuard R&D, Inc., who specializes in sustained release of bioactive compounds. This presentation discusses the repellent formulations and the preliminary use of the sustained release formulations in an environmentally sensitive area of a military base at Ft. Hood, TX. It has been documented through the Ft. Hood Endangered Species Program that significant predation occurs on the eggs and nestlings of the migratory Black-Capped Vireo and Golden-cheeked Warbler. The top two predators are snakes and the red imported fire ant, Solenopsis *invicta* (RIFA). The goal of this initial field study was to identify problem areas and assess the performance and of sustained release RIFA repellent systems in repelling RIFA away from nests and nestlings, thus increasing the probability for nestling survival. The sustained release formulations provided several months repellent activity under laboratory conditions. Similar formulations were prepared for use in preventing fire ants from foraging in scrub bushes harboring black-capped vireo nests. In this preliminary experiment of five nests that may have been predated on by fire ants only one was a treatment and four were in the controls. Additional work is planned this spring using a larger number of replicates.

LAND USE, ABUNDANCE OF *SOLENOPSIS INVICTA*, AND SPECIES DIVERSITY

Ken R. Helms & S. Bradleigh Vinson

Department of Entomology, Entomology Research Laboratory, Texas A&M University, College Station, Texas 77843-2475

khelms@neo.tamu.edu

INTRODUCTION

It is well known that the size of *Solenopsis invicta* populations can differ greatly from place to place; however, we know very little about the factors responsible. One obvious difference in abundance occurs between open areas and woodlands (e.g. Tschinkel 1988). However, why population size varies from place to place within seemingly similar habitats is largely unstudied. One approach is to first determine whether similar habitats differ consistently in *S. invicta* abundance according to site-specific differences. We employ this approach by comparing the population size of *S. invicta* in two similar, yet distinct, habitats occurring in close proximity in east Texas.

METHODS

The study was conducted on seven sites used in cattle grazing (cattle pastures), and seven undisturbed non-agricultural sites (old fields). Each site had remained in its current use for at least 10 years prior to the study. All were open (non-wooded) habitats, dominated by grasses and forbs. To equalize differences among site types related to the physical environment, they were selected as pairs (one old field, one cattle pasture) in close proximity. Because undisturbed non-wooded sites are infrequent in our region, we first located an old field and then the nearest available cattle pasture. Six of seven site pairs were directly adjacent, separated by a road or fence. The remaining site pair was separated by 7 km (two were unable to gain access a cattle pasture adjacent to the old-field site). Six of the seven site pairs were located in Brazos County, Texas, while one was located in Washington County, Texas.

Three 30 x 30 m sample plots were located within each site. Mean distance between plots per site was 0.196 ± 0.048 km (old fields) and 0.201 ± 0.023 km (cattle pastures). Within each plot, ground litter was collected from two randomly selected 1 x 1 m areas and pooled; ants were extracted using a Berlese funnel. Ants were also collected with four pitfall traps per plot (9 cm diameter x 12 cm height plastic cups) placed equidistantly in a grid at 10 m intervals. Traps were set in the field, filled with soil, and left for one week to acclimatize ants to their presence, then the soil was removed, and ethylene glycol was added. Fifteen days later, the traps were collected and returned to the lab, where all ants were removed. Ants within pitfall and litter samples were sorted, identified to species and counted. For statistical purposes, there were five samples per plot, four from pitfall traps and one from ground litter.

RESULTS

Sixteen ant species were found in old-field sites, while only three were found in cattle pastures. Fourteen of 16 old-field species were native; one of three species in cattle pastures was native. Regardless of site type, *S. invicta* was significantly more abundant than all other ants combined (> 95% were *S. invicta* in both habitats; Fig. 1); however, in cattle pastures, *S. invicta* was significantly more abundant than in old fields and species diversity was significantly less (Fig. 1, Fig. 2). In addition, increased abundance of *S. invicta* was correlated with decreasing abundance of other ant species (all plots (*N* = 42): *r* = -0.456, *P* < 0.005; old fields (21 plots): *r* = -0.571, *P* < 0.01; cattle pastures (21 plots): *r* = -0.155, *P* > 0.4 (*NS*), Spearman Rank Correlations).

DISCUSSION

Our study shows that the abundance of *S. invicta* and coexisting species can depend greatly upon land management practices, although the reason remains unclear. It is possible that cattle pastures provide key resources that allow *S. invicta* to reach exceptional densities, which results in particularly strong negative effects on other ant species. Because the habitats we studied differ primarily in their biotic communities, key resources for *S. invicta* in grazing pastures could include something related directly to cattle, or, perhaps more likely, something related to the plant community. In our study, old fields exhibit much greater plant species diversity, with grazing pastures generally dominated by a small number of introduced grasses. One of the most common of these is bermudagrass (*Cynodon dactylon*), a preferred species for grazing cattle, and a major host plant of an invasive mealybug, *Antonina graminis*. Honeydew from this mealybug appears to be an important nutritional resource for *S. invicta* in the southeastern United States (Helms and Vinson 2003), and it could be an important factor in explaining the difference in abundance of *S. invicta* we found.
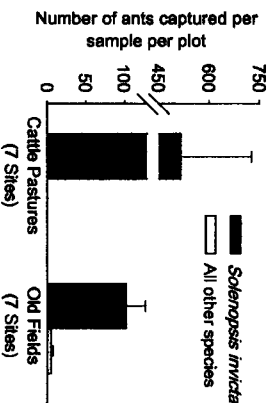
Number of ants captured per sample per plot

Figure 1. *Solenopsis invicta* is significantly more abundant in cattle pastures than in old fields (*P* < 0.05, Kruskal-Wallis anova), while other ants are significantly more abundant in old fields than cattle pastures (*P* < 0.001, Kruskal-Wallis anova)
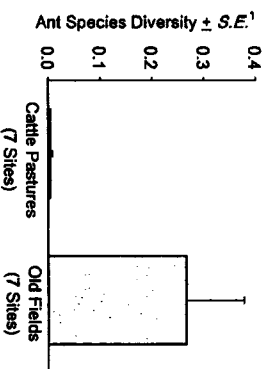

[1] Shannon diversity index

Figure 2. Ant species diversity is much greater in old fields than in cattle pastures (*P* < 0.005, Kruskal-Wallis Anova).

Our finding that there was a negative correlation between the abundance of *S. invicta* and native species, as well as a negative association between *S. invicta* abundance and ant species diversity (between habitat types), may appear at odds with a recent study where ant species richness and abundance were positively correlated with *S. invicta* abundance (Morrison and Porter 2003). The difference between our results and those of Morrison and Porter (2003) may be attributed to at least two possible factors. First, the majority of *S. invicta* studied by Morrison and Porter (2003) were monogyne, while polygyne colonies appear most frequent in our region of Texas. The polygyne form can reach greater densities and have greater impacts on other species. Second, positive correlations between the abundance of *S. invicta* and ant species diversity might occur because common environmental factors influence the success of both *S. invicta* and other ant species (Morrison and Porter 2003). Because of our site selection procedure, there was, on average, little environmental variation between sites other than those related to land use pattern. However, the study of Morrison and Porter (2003) could have encompassed greater environmental variation. At one level (across a variety of environments), species diversity could be affected largely by suitability of the environment, while, at another level (within similar environments), it could be largely affected by competitive interactions. The negative effects of *S. invicta* on native species (when they occur) could be obscured in analyses encompassing substantial environmental variability.

REFERENCES CITED

Helms, K.R. and S. B. Vinson. 2003. Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. Ecology 83: 2425-2438.

Morrison, L.W. and S.D. Porter. 2003. Positive association between densities of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and generalized ant and arthropod diversity. Environmental Entomology 32: 548-554.

Tschinkel, W. R. 1988. Distribution of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) in northern Florida in relation to habitat and disturbance. Annals of the Entomological Society of America 81: 76-81.

ACKNOWLEDGEMENTS

Appendix B

Papers/Posters Presented but not Submitted for Inclusion

Discovery and Characterization of Viruses in the Imported Fire Ant, *Solenopsis invicta.*
Steven Valles. USDA/ARS

The Evaluation and Release of Three New Fire Ant Decapitating Flies.
Sanford D. Porter and Ricardo J. Vazquez. USDNARS-CMAVE Juan A. Briano and
Luis A. Calcaterra. USDA/ARS-SABCL

Mammalian Cardiovascular & Neurologic Responses Elicited by Synthetic Alkaloids from
Solenopsis invicta (Imported Fire Ant) Venom. George Howell, Jeremy Gibson,
David McClendon, D. Nanayakkura, G-B   Yi, and Robin Rockhold. University of Mississippi
Medical Center and University of Mississippi

Final Report on Fire Ant IPM Study on DOD Facilities in South Carolina.
David F. Williams. USDNARS-CMAVE

Fire Ant Attacks on Patients in Nursing Homes: An Increasing Problem.
Robin Rockhold, R.D. deShazo, S.F. Kemp, M.D. deShazo, J. Goddard.
University of Mississippi Medical Center and Mississippi Department of Health

Cleveland County, Arkansas - Neighborhood Demonstration of Fire Ant Management.
Les Walz, Donna Shanklin, Kelly Loftin, and John Hopkins. UA-CES

Calhoun County, Arkansas – 3 Communities, 3 Fire Ant Management Efforts.
Alan Lee, Donna Shanklin, Kelly Loftin, and John Hopkins. UA-CES

Release of *Pseudacteon curvatus* in South Carolina. Timothy Davis, Clemson University

Appendix C

AMDRO® PRO Fire Ant Bait

Got Fire Ants on your turf?

The AMDRO defense is your best line of defense against fire ants.

It's easy. Broadcast AMDRO Yard Treatment throughout your entire yard because treating your whole yard attacks both visible and hidden mounds. And, the only way to treat a mound you can t see is to treat the entire yard.

It's effective. AMDRO starts to work immediately because worker ants think AMDRO is food. They pick it up and run with it straight back to the mounds, feeding AMDRO to the other ants and to the queen. It kills the queen, fast, prevents moving mounds",

and provides season long control!

**NO MORE QUEEN.
NO MORE MOVING MOUNDS.
NO MORE FIRE ANTS.**

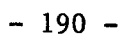No other brand can deliver all this.

AMDRO. Kills the queen, fast!

Season Long Control

The AMDRO DEFENSE
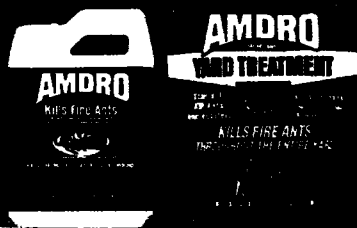
AMDRO
Kills Fire Ants

AMDRO
YARD TREATMENT
KILLS FIRE ANTS

Fireproof your world.

Eliminate the threat of *fire ants* for up to a full year with one broadcast application.

You have a choice for fire ant treatment. One that works better, lasts longer and is easier to use than the others. It's soon to be the top choice for fire ant control, so we're calling it just that—TopChoice!™

Get rid of existing fire ants in one step. TopChoice simplifies fire ant control: No multi-step treatment processes. No time-consuming mound treatments. And just one broadcast application of the ultra-low-dose granule can achieve 95 percent control in 4 to 6 weeks.

Prevent reinfestations for up to a year. Once fire ants are gone, expect them to stay gone for up to a full year. TopChoice has the residual power to control both developing queen cells and new queens that enter the landscape over the next 52 weeks. It's a novel concept in fire ant control: *prevention.*

Forget about mole crickets, too. The active ingredient in TopChoice (fipronil) is more than just a top-notch fire ant product. It's also the world's best mole cricket control. So while your fire ant problem is fading away, your mole crickets will be, too.

Don't be burned again by fire ant treatments that are more trouble than they're worth. Choose something different. Really different.

Learning all about the possibilities TopChoice presents and how to make this innovative fire ant control work for you requires that you talk with an authorized TopChoice agent. For more information on TopChoice, and for the agent nearest you, call 800-331-2867 or visit www.BayerProCentral.com.
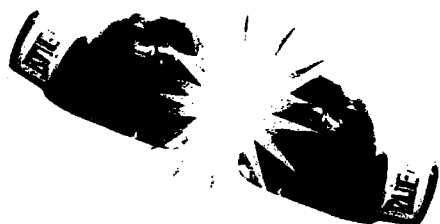
topchoice™

RIFARID
RED IMPORTED FIRE ANT TREATMENT TECHNOLOGIES

A new method for 100% kill of RIFA colonies with patent-pending "knockout" technology

The Red Imported Fire Ant is aggressive and resilient. Many contact treatments just spread the problem around - the ants sense the treatment and escape to form new mounds.

RIFARID's non-toxic "knockout" technology instantly puts the insects to sleep, and works with all varieties of contact insecticides to dramatically increase their effectiveness.

Visit our booth at the Annual Red Imported Fire Ant Conference In Baton Rouge, Louisiana, March 21-23, 2004!

Contact: Tom Taylor, Founder and President
RIFARID LLC; Lake Charles, LA
www.rifarid.com   info@rifarid.com
Tom Taylor (337) 477-8190   Tim Taylor (303) 641-5389

**Only Extinguish® Plus Gives 'em Both Barrels.**

Fast acting. Long lasting. Extinguish Plus is the only dual active bait that kills adult fire ant workers and sterilizes the queen.

- Blow away fire ants.

- They account for nearly $6 billion* in losses annually in the U.S.

- Extinguish Plus gives you double barrels—an adulticide to knock 'em down and an IGR for long-lasting control.

- It's fire ant ammo approved for indoor and outdoor use where fire ants are a problem.

Aim for superior results, reduced costs and dead fire ants. For more information, call us at 1-800-248-7763.

In your area, contact H.F. Warns, Regional Manager, at 1-800-877-6374, ext. 6443.

www.wellmarkinternational.com
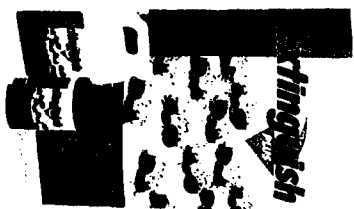
Appendix D

"Chip" Anderson
Bayer Environmental Science
805 S. Glynn Street
Fayetteville, GA 30214
chip.anderson@bayercropscience.com

James Anderson
Center for Wetland & Water Resources
University of Mississippi
15 Road 2075
Abbeville, MS 38601
jandersn@olemiss.edu

Jeffrey Avison
Ambrands
2255 Cumberland Pkwy.
Building 600-B
Atlanta, GA 30339
javison@ambrandsproducts.com

Jack Baldwin
LSU AgCenter
400A Life Sciences Building
Baton Rouge, LA 70803
jbaldwin@agctr.lsu.edu

Casey Barocco
LSU AgCenter
404 Life Sciences Building
Dept. of Entomology
Baton Rouge, LA 70803
cbarocco@juno.com

Charles Barr
Texas Cooperative Extension
P.O. Box 2150
Bryan, TX 77806
c-barr@tamu.edu

Vicky Bertagnolli
Auburn University
Dept. of Entomology and Plant Pathology
301 Funchess Hall
Auburn University, AL 36849
bertave@acesag.auburn.edu

Allan Beuerman
Cooperative Ext. Services
University of Arkansas
2901 W. Roosevelt Rd.
Little Rock, AR 72204

Allan Beuerman
Cooperative Extension Service
University of Arkansas
2901 W. Roosevelt Road
Little Rock, AR 72204
abeuerman@uaex.edu

Herb Bolton
USDA, CSREES
Washington, DC 20250
hbolton@csrees.usda.gov

Charles Brown
USDA/APHIS
4700 River Rd. Unit 134
Riverdale, MD 20737
charles.l.brown@aphis.usda.gov

Kenneth Calcote
1033 Hwy 82, E. Lot #2
Starkville, MS 39759

Alejandro Calixto
Texas Cooperative Extension
Department of Entomology
College Station, TX 77843
acalixto@tamu.edu

Anne-Marie Callcott
USDA, APHIS, PPQ, CPHST, GPPS
3505 25th Ave, Bldg 16
Gulfport, MS 39501
anne-marie.a.callcott@aphis.usda.gov

Michael Chapman
Bayer Environmental Science
1856 Spahn Lane
Placentia, CA 92870
mike.chapman@bayercropscience.com

Jian Chen
USDA-ARS
PO Box 67
Stoneville, MS 38776
jianchen@ars.usda.gov

Stacy Clayton
LSU Agricultural Center
404 Life Sciences Bldg
Dept of Entomology
Baton Rouge, LA 70803
sclayton@agctr.lsu.edu

Dee Colby
LSU Agricultural Center
404 Life Sciences Bldg
Dept of Entomology
Baton Rouge, LA 70803
dcolby@lsu.edu

Jeanetta Cooper
Oklahoma Dept. of Ag Food & Forestry
PO Box 528804
Oklahoma City, OK 73152
jcooper@oda.state.ok.us

Timothy Davis
Clemson University
P.O. Box 23205
Columbia, SC 29016
tdvs@clemson.edu

Charles Dawson
University of Mississippi
5 CR 302
Oxford, MS 38655

Stan Diffie
University of Georgia
Department Of Entomology
PO Box 748
Tifton, GA 31793
diffie@tifton.uga.edu

Bart Drees
Texas Cooperative Extension
2475 TAMU
College Station, TX 77843
diffie@tifton.uga.edu

Richard Driscoll, Sr.
Driscoll Products
225 Rambling Loop
Weatherford, TX 76087
llocsird@earthlink.net

Jimmy Dunkley
La. Dept. of Agriculture & Forestry
5825 Florida Blvd
Baton Rouge, LA 70806
jimmy_d@ldaf.state.la.us

Lee Eisenberg
LSU Agricultural Center
404 Life Sciences Bldg
Baton Rouge, LA 70803
leisenberg@agcenter.lus.edu

Mary Elias
Fire Ant Specialist, LLC
110 Irwin St
Pickens, SC 29671
maelias@innova.net

Kim Engler
Texas Cooperative Extension
17360 Coit Road
Dallas, TX 75252
K-engler@tamu.edu

Hugh Ettinger
Hugh Ettinger Associates, Ltd
1028 Constantinople St.
New Orleans, LA 70115
hettinger@hindspring.com

Henry Fadamiro
Auburn University Dept. of Entomology
301 Funchess Hall
Auburn, AL 36830

Kevin Fincannon
Fire Ant Specialist, LLC
110 Irwin Street
Pickens, SC 29671

Kathy Flanders
Auburn University
208A Extension Hall
Auburn, AL 36849
kflander@aces.edu

Jason Forster
Auburn University Dept of Entomology
301 Funchess Hall
Auburn, AL 36830
forstja@auburn.edu

John Fortino
Bayer Environmental Science
2675 Ashmont Dr.
Germantown, TN 38138
john.fortino@bayercropscience.com

Bart Fox
Syngenta Crop Protection, Inc.
3302 Walnut Cove Court
Friendswood, TX 77546
bart.fox@syngenta.com

Michael French
University of Arkansas Coop Ext
P.O. Box 391
Little Rock, AR 72203
mfrench@uaex.edu

James Fuxa
LSU Agricultural Center
404 Life Sciences Bldg
Baton Rouge, LA 70803
jfuxa@lsu.edu

Mel Garbett
Ambrands
12657 Alcosta Blvd, Suite 175
San Ramon, CA 94583
garbmel@aol.com

Wayne Gardner
University of Georgia, Dept of Entomology
1109 Experiment Street
Griffin, GA 30223
wgardner@griffin.uga.edu

William Glenn
Clemson University
511 Westinghouse Road
Pendleton, SC 29670
kglenn@clemson.edu

Bucky Godbold
Fire Ant Control
3703 Pine Haven Drive
Florence, SC 29506
dlobdog@aol.com

Bryan Gooch
Bayer Corporation
9527 Foggy Meadow Road
Charlotte, NC 28269

Jerry Goolsby
Valent BioSciences
2232 Stonegate
Denton, TX 76205
jerry.goolsby@valent.com

Lawrence Fudd Graham
Auburn University, Dept of Entomology
301 Funchess Hall
Auburn, AL 36849
fgraham@acesag.auburn.edu

Bradley Guice
P.O. Box 790
Winnsboro, LA 71295

Roger Hasse
University of Mississippi/NCPA
1 Coliseum Drive
University, MS 38677
rhasse@olemiss.edu

Gregg Henderson
LSU Ag Center, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
GRHenderson@agcenter.lsu.edu

Don Henne
LSU Ag Center, Dept. of Entomology
404 Life Sciences Bldg
Baton Rouge, LA 70803
dhenne@agcenter.lsu.edu

David Herd
Herd Seeder Company, Inc.
PO Box 448
Logansport, IN 46947
info@herdseeder.com

Linda Hooper-Bui
LSU Ag Center, Dept. of Entomology
404 Life Sciences Building
Baton Rouge, LA 70803
lhooper@agctr.lsu.edu

John Hopkins
University of Arkansas, Dept. of Entomology
PO Box 391
Little Rock, AR 72203
jhopkins@uaex.edu

Paul (Mac) Horton
Clemson University Extension
P.O. Box 23205
Columbia, SC 29224
mhorton@clemson.edu

George (Trey) Howell, III
University of Mississippi Medical Center
Dept. of Pharmacology & Toxicology
2500 North State Street
Jackson, MS 39216
ghowell@pharmacology.umsmed.edu

Rong Hu
Texas A&M University
College Station, TX 77843
r-hu@tamu.edu

Lacy Inmon
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
linmon@lsuagcenter.edu

Zhisheng Jiang
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
zjiang@agcenter.lsu.edu

Bruce Johnson
Clemson University
Harry County Extension Office
Conway, SC 29526
bjhnsn@clemson.edu

Seth Johnson
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
sjohnson@agctr.lsu.edu

Tracy Johnson
DEMCO
P.O. Box 15659
Baton Rouge, LA 70895
tracyj@demco.org

Kathie Kalmowitz
BASF Specialty Products
26 Davis Drive
Research Triangle Park, NC 27709
kalmowk@basf.com

Vedham Karpakakunjaram
Oklahoma State University
Dept. of Entomology
127 Nobel Research Center
Stillwater, OK 74078-3033
karpaka@okstate.edu

Creig Kimbro
University of Tennessee
Ag Extension Service
P.O. Box 338
Coalmont, TN 37313
ckimbro@utk.edu

Keri Landry
LSU School of Renewable Resources
Baton Rouge, LA 70803
klandr5@lsu.edu

Curtis Lard
Texas A&M University
Dept. of Ag Economics
2124 TAMU
College Station, TX 77843
c-lard@tamu.edu

Alan Lee
University of Arkansas
Cooperative Extension Service
First and Hunt Box 38
Hampton, AR 71744
alee@uaex.edu

Nannan Liu
Auburn University
610 Lockwood Street
Auburn, AL 36830
nlin@acesag.auburn.edu

Kelly Loftin
2301 South University Avenue
Little Rock, AR 72203
kloftin@uaex.edu

Evangeline Long
Clemson University
1555 Richland Ave East, Suite 500
Aiken, SC 29801
elattim@clemson.edu

John Long
FMC Corporation
Philadelphia, PA 19103
john_long@fmc.com

David Madison
Arizona Dept. of Agriculture
1688 West Adams
Phoenix, AZ 85007
david.madison@agric.state.az.us

Charles Mason
Auburn University
Cooperative Extension Systems
Auburn, Alabama 36849

Lee McAnally
USDA, APHIS, PPQ, CPHST, GPPS
3505 25th Avenue, Bldg. 16
Gulfport, MS 39501
lee.r.mcanally@aphis.usda.gov

Foster McWhorter
Bayer Environmental Science
Bullard, TX 75757
fostermcwhorter@flash.net

Maynard Milks
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg
Baton Rouge, LA 70803
mmilks1@lsu.edu

Wayne Mixson
The Scotts Company
P.O. Box 2187
Apopka, FL 32704
wayne.mixson@scotts.com

Don Molino
Louisiana Agri-News Network
263 Third Street, 5th Floor
Baton Rouge, LA 70801
Donthefarmboy@yahoo.com

Esther Mwangi
University of Mississippi
1 Coliseum Drive
University, MS 38677
emwangi@olemiss.edu

Roger Nelson
Carl Albert State College
1507 S. McKenna
Poteau, OK 74953
rnelson@carlalbert.edu

Paul Nester
Texas Cooperative Extension
#2 Abercrobmie Drive
Houston, TX 77084
p-nester@tamu.edu

David Oi
USDA-ARS, CMAVE
1600-1700 SW 23rd St.
Gainesville, FL 32604
doi@gainesville.usda.ufl.edu

Jason Oliver
TSU Nursery Crop Research Station
472 Cadillac Lane
McMinnville, TN 37110
joliver@tnstate.edu

Jack Orman
Bengal Products, Inc.
P.O. Box 40487
Baton Rouge, LA 70835
jorman@bengal.com

J. Patrick Parkman
University of Tennessee
205 Ellington Plant Sciences Bldg.
2431 Center Drive
Knoxville, TN 37996
jparkman@utk.edu

Roberto Pereira
USDA-ARS, CMAVE
1600-1700 SW 23rd Dr
Gainesville, FL 32604
rpereira@gainesville.usda.ufl.edu

Douglas Petty
University of Arkansas
Cooperative Extension Service
400 Laurel, Suite 215
Texarkana, AR 71854
dpetty@uaex.edu

Dale Pollet
LSU AgCenter, Dept. of Entomology
400A Life Sciences Bldg.
Baton Rouge, LA 70803
dpollet@agctr.lsu.edu

Sanford Porter
USDA-ARS, CMAVE
1600-1700 SW 23rd Drive
Gainesville, FL 32604
sdp@nersp.nerdc.ufl.edu

Steve Powell
Tennessee Dept. of Ag Regulatory Services
Box 40627, Melrose Station
Nashville, TN 37204
steve.powell@state.tn.us

Gregg Powers
Ambrands
2255 Cumberland Pkwy., Bldg 600-B
Atlanta, GA 30339
gpowers@ambrandsproducts.com

Anthony Pranschke
USDA/ARS
P.O. Box 67
Stoneville, MS 38776
apranschke@ars.usda.gov

Gary Probst
Ambrands
2255 Cumberland Pkwy, Bldg 600-B
Atlanta, GA 30339
gprobst@ambrandsproducts.com

Robert Puckett
Texas A&M University, Dept, of Entomology
2475 TAMU
College Station, TX 77843
rpuck@tamu.edu

Tahir Rashid
University of Tennessee
472 Cadillac Lane
McMinnville, TN 37110
rashid@blomand.net

Neil Reimer
Hawaii Department of Agriculture
Plant Quarantine Branch
1849 Auiki Street
Honolulu, HI 96819
neil.j.reimer@hawaii.gov

James Reinert
Texas A&M University
Texas Agricultural Experiment Station
17360 Coit Rd
Dallas, TX 75252
j-reinert@tamu.edu

Stanley Rice
Southeastern Oklahoma State University
1405 N. 4th Ave, PMB 4008
Durant, OK 74701
srice@sosu.edu

Arthur Richter
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
arichter@agctr.lsu.edu

Claudia Riegel
New Orleans Mosquito & Termite
1613 Lucas Place
Baton Rouge, LA 70816

Nathan Riggs
Texas Cooperative Extension
3355 Cherry Ridge, Suite 212
San Antonio, TX 78230
n-riggs@tamu.edu

Robin Rockhold
University of Mississippi Medical Center
Dept. of Pharmacology & Toxicology
2500 N. State Street
Jackson, MS 39216
rrockhold@pharmacology.umsmed.edu

Ronald Rosenberg
USDA, ARS, NPS
5601 Sunnyside Avenue
Bldg. 4, Room 2178
Beltsville, MD 20705
rmr@ars.usda.gov

Jessica Rosson
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
jrosson@lsu.edu

Carol Russell
USDA/APHIS/PPQ
300 Ala Moana Blvd #8-152
Honolulu, HI 96850
carol.e.russell@aphis.usda.gov

Clay Scherer
DuPont Professional Products
PO Box 30 Elkton Road
Newark, DE 19714
clay.w.scherer@usa.dupont.com

Tim Schowalter
LSU AgCenter, Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
tschowalter@agctr.lsu.edu

Donna Shanklin
Ext Entomologist-Fire Ants, SEREC
P.O. Box 3508
Monticello, AR 71656
shanklin@uamont.edu

Charles Silcox
DuPont Professional Products
Wilmington, DE 19880
charles.a.silcox@usa.dupont.com

Steven Sims
Whitmire Micro-Gen Research Labs, Inc.
3568 Tree Court Industrial Blvd
St. Louis, MO 63122
steve.sims@jwp.com

Fred Singleton
Clemson University
Dept. of Plant Pathology
Summerville, SC 29483
fsngltn@clemson.edu

Wayne Smith
Oklahoma State University
PO Box 749
Durant, OK 74702
smithwa@okstate.edu

Yuliya Sokolova
LSU Dept. of Entomology
404 Life Sciences Bldg.
Baton Rouge, LA 70803
jumicro@yahoo.com

Christopher Stafford
Quant Technologies, PTY LTD
Cooroy, QLD Australia 4563
chris@email.tc

Douglas Streett
USDA/ARS, BCPRU
PO Box 67
Stoneville, MS 38776
dstreett@ars.usda.gov

Carrick Talmadge
University of Mississippi
45A CR 224
Oxford, MS 38655

Sara Taylor
RIFARID LLC
Austin, TX 78704
cyntay@austin.rr.com

Timothy Taylor
RIFARID LLC
4011 Creole Street
Lake Charles, LA 70605
taylor_tim@hotmail.com

Tommy Taylor
RIFARID LLC
4011 Creole Street
Lake Charles, LA 70605
emotay@aol.com

Larry Thead
USDA/ARS, BCPRU
Mississippi State, MS 39762
lthead@msa-msstate.ars.usda.gov

Jamie Trotter
Louisiana State University at Alexandria
8100 Hwy. 71 South
Alexandria, LA 71302
egossett@lsua.edu

A. Turner
Bayer Environmental Science
Jacksonville, FL 32259
al.turner@bayercropscience.com

Karen Vail
University of Tennessee
205 Ellington Plant Science Bldg.
2431 Parker Drive
Knoxville, TN 37996
kvail@utk.edu

Steven Valles
USDA/ARS
1600 SW 23rd Drive
Gainesville, FL 32608
svalles@gainesville.usda.ufl.edu

David Vander Hooven
Advanced Organics, Inc.
1124 Fort Street
Maumee, OH 43537
byprodvh@accesstoledo.com

Doug VanGundy
Wellmark International
12200 Denton Drive
Dallas, TX 75234
doug.vangundy@wellmarkint.com

S. Bradleigh Vinson
Texas A&M University, Dept. of Entomology
2475 TAMU
College Station, TX 77843
bvinson@neo.tamu.edu

James Vogt
USDA-ARS-BCPRU
PO Box 67
Stoneville, MS 38776
jvogt@ars.usda.gov

Les Walz
University of Arkansas
Cooperative Extension Service
501 E. Magnolia, Box 386
Rison, AR 71665
lwalz@uaex.edu

Kenneth Ward
Alabama A&M University
Center for Forestry & Ecology
PO Box 1208
Normal, AL 35762
kenneth.ward@email.aamu.edu

Rufina Ward
Alabama A&M University
Center for Forestry & Ecology
PO Box 1208
Normal, AL 35762
rward@aamu.edu

Anne-Marie Wiese
Bayer CropScience
5017 Andover Drive
Plano, TX 75023
ann.wiese@bayercropscience.com

David Williams
USDA-ARS, CMAVE
10012 NW 62nd Lane
Gainesville, FL 32653
dfwilliams@ifas.ufl.edu

Michael Williams
Auburn University
Dept. of Entomology and Plant Pathology
301 Funchess Hall
Auburn University, AL 36849
dfwilliams@ifas.ufl.edu

Beverly Wiltz
University of Georgia, Dept of Entomology
1109 Experiment Street
Griffin, GA 30223
bwiltz@uga.edu

Russell Wright
Oklahoma State University
Stillwater, OK 74078
rew0675@okstate.edu

Julian Yates, III
University of Hawaii at Manoa
Dept. of Entomology
3050 Maile Way, Room 310
Honolulu, HI 96822
yates@hawaii.edu

Clete Youmans
BASF
1875 Viar Road
Dyersburg, TN 38024

Nadeer Youssef
Tennessee State University
McMinnville, TN 37110
nyoussef@tnstate.edu